BIOLOGY

Laboratory Manual

Eleventh Edition

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Mc Graw Hill Education

Biology

Laboratory Manual

eleventh edition

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BIOLOGY LABORATORY MANUAL, ELEVENTH EDITION

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Some of the laboratory experiments included in this text may be hazardous if materials are handled improperly or if procedures are conducted incorrectly. Safety precautions are necessary when you are working with chemicals, glass test tubes, hot water baths, sharp instruments, and the like, or for any procedures that generally require caution. Your school may have set regulations regarding safety procedures that your instructor will explain to you. Should you have any problems with materials or procedures, please ask your instructor for help.

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Contents

Indicates a *LearnSmart Lab*® activity is available for all or part of this exercise. For more information, visit www.learnsmartadvantage.com.

Preface

We have designed this laboratory manual for an introductory biology course with a broad survey of basic laboratory techniques. The experiments and procedures are simple, safe, easy to perform, and especially appropriate for large classes. Few experiments require more than one class meeting to complete the procedure. Each exercise includes many photographs, traditional topics, and experiments that help students learn about life. Procedures within each exercise are numerous and discrete so that an exercise can be tailored to the needs of the students, the style of the instructor, and the facilities available.

TO THE STUDENT

We hope this manual is an interesting guide to many areas of biology. As you read about these areas, you'll probably spend equal amounts of time observing and experimenting. Don't hesitate to go beyond the observations that we've outlined – your future success as a scientist and an informed citizen depends on your ability to seek and notice things that others may overlook. Now is the time to develop this ability with a mixture of hard work and relaxed observation. Have fun, and learning will come easily. Also, remember that this manual is designed with your instructors in mind as well. Go to them often with questions – their experience is a valuable tool that you should use as you work.

TO THE INSTRUCTOR

This manual's straightforward approach emphasizes experiments and activities that optimize students' investment of time and your investment of supplies, equipment, and preparation. Simple, safe, and straightforward experiments are most effective if you interpret the work in depth. Most experiments can be done easily by a student in 2 to 3 hours. Terminology, structures, photographs, and concepts are limited to those that the student can readily observe and understand. In each exercise we have included a few activities requiring a greater investment of effort if resources are available, but omitting them will not detract from the objectives.

This manual functions best with an instructor's guidance and is not an autotutorial system. We've tried to guide students from observations to conclusions, to help students make their own discoveries, and to make the transition from observation to understanding biological principles. But discussions and interactions between student and instructor are major components of a successful laboratory experience. Be sure to examine the "Questions for Further Thought and Study" in each exercise. We hope they will help you expand students' perceptions that each exercise has broad application to their world.

KEY UPDATES TO THE 11TH EDITION

DIGITAL INTEGRATION



As educators, we recognize that today's students are digital learners. Therefore, a significant feature of this edition is the integration of various digital resources into the content of the exercises. Virtually every exercise of this manual is now accompanied by tailor-made digital resources. Rather than generic images from Internet sources, we have produced a variety of high-definition videos, PowerPoint images, and other resources that demonstrate basic techniques, emphasize biological principles, test for understanding, and engage students as they learn biology in the laboratory. For this edition, we have included numerous new videos.

All digital resources can be found at **connect.mheducation.com.** Students will enjoy viewing these presentations, and instructors will want to assign these resources to help students know what they'll be doing, what principles they'll be investigating, and what concepts they'll need to understand before coming to lab.

EXERCISE UPDATES

Updates and changes include:

- Yellow highlighting is placed throughout the manual to indicate, at a glance, all laboratory actions expected of the students.
- Many procedures are revised to help clarify steps and outline what needs to be done.
- An extensive number of photographs are added or changed to improve your understanding and provide a visual of what you will see.
- Additional art is added to help illustrate concepts, procedures, and results.
- New boxed inserts highlight the relationships of biological processes with health care.

As you examine this manual, you'll see that we've improved several of the most popular and effective features of previous editions:

- **Safety First** and **Caution** icons remind you to read the manual to ensure that you are aware of safety issues associated with the exercises.

- **Learning Objectives** will give you an overview of what you will do and learn in the exercise.
- **Writing to Learn Biology** will encourage you to expand on what you have done and learned.
- **Investigations, Procedures,** and **Doing Biology Yourself** will require you to *do* biology and apply skills you've learned to develop and answer your own questions about biology.
- **Questions** throughout each exercise will encourage you to integrate and reflect on what you've done and learned.
- **Questions for Further Thought and Study** at the end of each exercise will help you apply what you've learned to other topics.

As noted previously, we have also tailored a variety of videos and other visual materials to help you succeed in the laboratory. You'll learn from a growing library of high-quality videos that demonstrate basic laboratory techniques. You'll observe these techniques in action before you polish your own skills in the laboratory.



Teaching and Learning Tools

McGraw-Hill Connect® Biology

McGraw-Hill Connect Biology provides online presentation, assignment, and assessment solutions. It connects your students with the tools and resources they'll need to succeed. **connect.mheducation.com**.

With Connect Biology, you can deliver assignments, quizzes, and tests online. A robust set of questions and activities are presented and aligned with the textbook's learning outcomes. Pre-lab worksheets and Investigation worksheets are also included within Connect. As an instructor, you can edit existing questions and write entirely new problems. Track students' performance—by question, assignment, or in relation to the class overall—with detailed grade reports. Integrate grade reports easily with Learning Management Systems (LMS), such as Blackboard—and much more.

LearnSmart Labs®



Based on the same adaptive technology as LearnSmart, LearnSmart Labs is an outcomes-based lab simulation that assesses a student's knowledge and adaptively corrects deficiencies, allowing the student to learn faster and retain more knowledge with greater success.

First, a student's knowledge is focused on core learning outcomes: Questioning reveals knowledge deficiencies that are corrected by the delivery of content that is conditional on a student's response. Then, a simulated lab experience requires the student to think and act like a scientist: Recording, interpreting, and analyzing data using simulated equipment found in labs and clinics. The student is allowed to make mistakes—a powerful part of the learning experience! A virtual coach provides subtle hints when needed, asks questions about the student's choices, and allows the student to reflect upon and correct those mistakes. Whether your need is to overcome the logistical challenges of a traditional lab, provide better lab prep, improve student performance, or make your online experience one that rivals the real world, LearnSmart Labs accomplishes it all. To learn more, visit **www.learnsmartadvantage.com**.

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Laboratory Resource Guide

The *Laboratory Resource Guide* is essential for instructors and laboratory assistants and is available free to adopters of the Laboratory Manual within Connect under the Instructor Resources tab.

Welcome to the Biology Laboratory

Welcome to the biology laboratory! Although reading your textbook and attending lectures are important ways of learning about biology, nothing can replace the importance of the laboratory. In lab you'll get hands-on experience with what you've heard and read about biology—for example, you'll observe organisms, do experiments, test ideas, collect data, and make conclusions about what you've learned. You'll do biology.

You'll enjoy the exercises in this manual—they're interesting, informative, and can be completed within the time limits of your laboratory period. We've provided questions to test your understanding of what you've done; in some of the exercises, we've also asked you to devise your own experiments to answer questions that you've posed. To make these exercises most useful and enjoyable, follow these guidelines:

THE IMPORTANCE OF COMING TO CLASS

Biology labs are designed to help you experience biology first-hand. To do that, you must attend class. If you want to do well in your biology course, you'll need to attend class and pay attention. To appreciate the importance of class attendance for making a good grade in your biology course, examine figure 1, which is a graph showing how students'

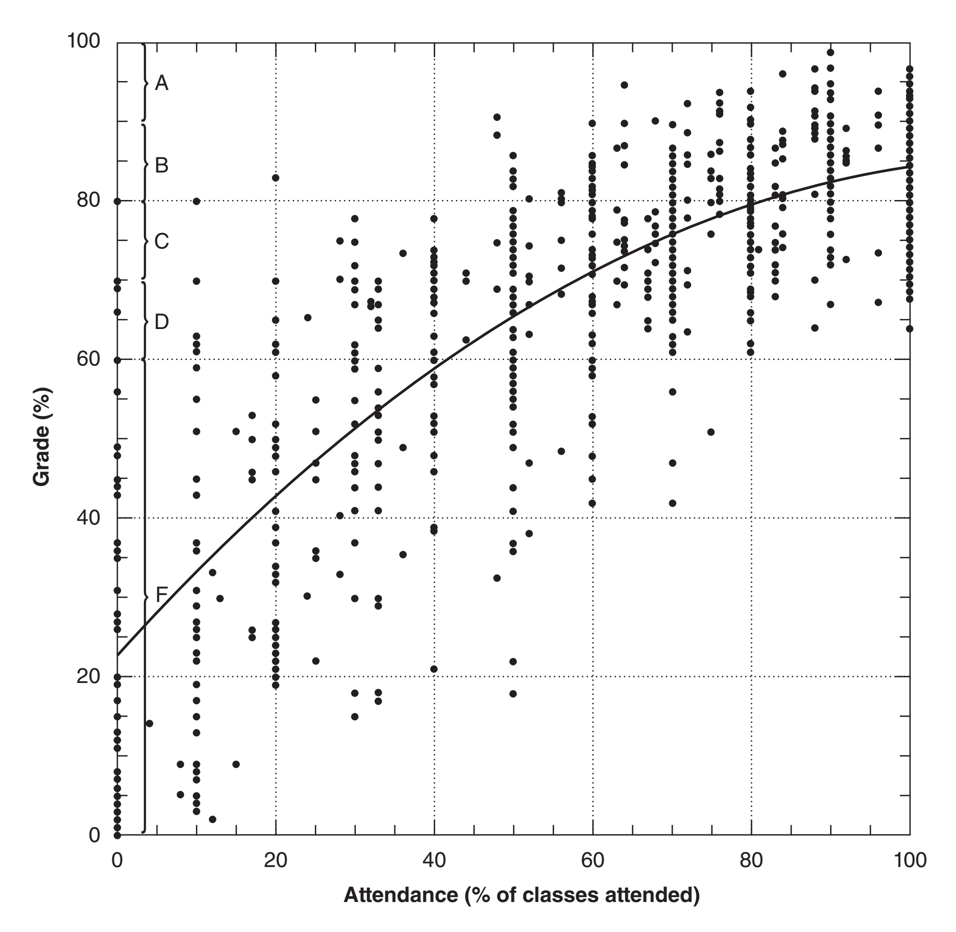


Figure 1 How students' grades in an introductory biology course relate to their rates of class attendance.

grades in an introductory biology course relate to their rates of class attendance. Data are from a general biology class at the University of Minnesota. On page xii, write an analysis of the data shown in figure 1. What do these data mean?

BEFORE COMING TO LAB

Watch the lab video. Videos are provided for several of the labs in this manual. Be sure to watch any video associated with the lab you will be completing. These videos will help you know more about what you will be doing, what principles you will be investigating, and what concepts you need to understand before coming to lab.

Read the exercise before coming to lab. This will give you a general idea about what you're going to do, as well as why you're going to do it. Knowing this will not only save time, it will also help you finish the experiments and make you aware of any safety-related issues associated with the lab.

Review any of the lab safety concerns. Before doing any procedures, you'll encounter a section of each exercise titled "SAFETY FIRST" that is marked with its icon:



This icon will warn you of safety concerns (e.g., solvents, acids, bases, hotplates) associated with the work. If you have questions about these safety issues, contact your lab instructor before starting the lab work.

Notify your instructor if you are pregnant, color-blind, taking immunosuppressive drugs, have allergies, or have any other conditions that may require precautionary measures. Also, before coming to lab, cover any cuts or scrapes with a sterile, waterproof bandage.

WHEN IN LAB

1. Know what you are going to do. Read and understand the lab before coming to lab.
2. Don't start the exercise until you've discussed the exercise with your laboratory instructor. She or he will give you specific instructions about the lab and tell you how the exercise may have been modified.
3. Work carefully and thoughtfully, and stay focused as you work. You'll be able to finish each exercise within the allotted time if you are well prepared and stay busy. You'll not be able to finish the exercise if you spend your time talking about this weekend's party or last week's big game.
4. Discuss your observations, results, and conclusions with your instructor and lab partners. Perhaps their comments and ideas will help you better understand what you've observed.
5. Always follow instructions and follow safety guidelines presented by your instructor.
6. If you have questions, ask your instructor.

SAFETY IN THE LABORATORY

Laboratory accidents can affect individuals, classes, or the entire campus. To avoid such accidents, the exercises in this manual were designed with safety as a top priority. You'll be warned about any potentially hazardous situations or chemicals with this image:



When you see this image, pay special attention to the instructions.

The laboratory safety rules listed in table 1 will help make lab a safe place for everyone to learn biology. Remember, it is much easier to prevent an accident than to deal with its consequences.

Read the laboratory safety rules listed in table 1. If you do not understand them, or if you have questions, ask your instructor for an explanation. Then complete table 1 and sign the statement that is at the bottom of page xii.

BEFORE YOU LEAVE LAB

Put away all equipment and glassware, and wipe clean your work area.

AFTER EACH LABORATORY

Soon after each lab, review what you did. What questions did you answer? What data did you gather? What conclusions did you make?

Also note any questions that remain. Try to answer these questions by using your textbook or visiting the library. If you can't answer the questions, discuss them with your instructor. Welcome to the biology laboratory!

Table 1

Laboratory Safety Rules

Rule	Why is this rule important? What could happen if this rule is not followed?
Behave responsibly. No horseplay or fooling around while in lab.	
Do not bring any food or beverages into lab, and do not eat, drink, smoke, chew gum, chew tobacco, or apply cosmetics when in lab. Never taste anything in lab. Do not put anything in lab into your mouth. Avoid touching your face, chewing on pens, and other similar behaviors while in lab. Always wear shoes in lab.	
Unless you are told otherwise by your instructor, assume that all chemicals and solutions in lab are poisonous, and act accordingly. Never pipette by mouth. Always use a mechanical pipetting device (e.g., a suction bulb) to pipette solutions. Clean up all spills immediately, and report all spills to your instructor.	
Wear safety goggles when working with chemicals. Carefully read the labels on bottles and know the chemical you are dealing with. Do not use chemicals from an unlabeled container, and do not return excess chemicals back to their container. Report all spills to your instructor immediately.	
Unless your instructor tells you to do otherwise, do not pour any solutions down the drain. Dispose of all materials as per instructions from your instructor.	
If you have long hair, tie it back. Don't wear dangling jewelry. If you are using open flames, roll up loose sleeves. Wear contact lenses at your own risk; contacts hold substances against the eye and make it difficult to wash your eyes thoroughly.	
Treat living organisms with care and respect.	
Your instructor will tell you the locations of lab safety equipment, including fire extinguishers, fire blanket, eyewash stations, and emergency showers. Familiarize yourself with the location and operation of this equipment.	
If anything is splashed into your eyes, wash your eyes thoroughly and immediately. Tell your lab instructor what happened.	
Notify your instructor of any allergies to latex, chemicals, stings, or other substances.	

Table 1

Laboratory Safety Rules (continued)

Rule	Why is this rule important? What could happen if this rule is not followed?
If you break any glassware, do not pick up the pieces of broken glass with your hands. Instead, use a broom and dustpan to gather the broken glass. Ask your instructor how to dispose of the glass.	
Unless told by your instructor to do otherwise, work only during regular, assigned hours when the instructor is present. Do not conduct any unauthorized experiments; for example, do not mix any chemicals without your instructor's approval.	
Do not leave any experiments unattended unless you are authorized by your instructor to do so. If you leave your work area, slide your chair under the lab table. Keep walkways and desktops clean and clear by putting books, backpacks, and so on along the edge of the room, in the hall, in a locker, or in an adjacent room. Keep your work area as clean and uncluttered as possible.	
Don't touch or put anything on the surface of hotplates unless told to do so. Many types of hotplates have no visible sign that they are hot. Assume they are hot.	
Know how to use the equipment in lab. Most of the equipment is expensive; you may be required to pay all or part of its replacement cost. Keep water and solutions away from equipment and electrical outlets. Report malfunctioning equipment to your instructor. Leave equipment in the same place and condition that you found it. If you have any questions about or problems with equipment, contact your instructor.	
Know what to do and whom to contact if there is an emergency. Know the fastest way to get out of the lab. Immediately report all injuries—no matter how minor—to your instructor. Seek medical attention immediately if needed. If any injury appears to be life-threatening, call 911 immediately.	
At the end of each lab, clean your work area, wash your hands thoroughly with soap, slide your chair under the lab table, and return all equipment and supplies to their original locations. Do not remove any chemicals or equipment from the lab.	

Name ____________________

Lab Section ____________________

Your lab instructor may require that you submit this page at the end of today's lab.

1. In the space below, write an analysis of the data shown in figure 1.

After completing table 1, read and sign this statement:

2. I have read and I understand and agree to abide by the laboratory safety rules described in this exercise and discussed by my instructor. I know the locations of the safety equipment and materials. If I violate any of the laboratory safety rules, my instructor will lower my grade and/or remove me from the lab.

____
Signature

____
Name (printed)

____
Date

LEARNSMART LABS BIOLOGY

Exercise 1

Scientific Method

The Process of Science

Learning Objectives

By the end of this exercise you should be able to:

1. Define science and understand the logic and sequence of the scientific method.
2. Develop productive observations, questions, and hypotheses about the natural world.
3. Calculate the range, mean, and standard deviation for a set of replicate measurements.
4. Design and conduct a controlled experiment to test a null hypothesis.
5. Understand the difference between a hypothesis and a scientific theory.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

The word *science* brings to mind different things to different students. To some students, science is a textbook. To others, it's a microscope, a dissected frog, or a course that you take. In fact, science is none of those things. Some definitions are more useful than others, but for biological research a good definition of **science** is *the orderly process of posing and answering questions about the natural world through repeated and unbiased experiments and observations*. This definition emphasizes that science is a process rather than a book, course, or list of facts. Science is not a "thing." It's a way of thinking about and doing things—a way of learning and knowing about the natural world (fig. 1.1).

Our definition also emphasizes that people do science by *asking questions* and then *doing experiments* to answer those questions. Questions and curiosity are part of human nature, and science is a human activity. Like any human task, it takes practice to do science effectively.

Finally, our definition emphasizes that science is a tool for learning about the *natural world*. It is ineffective for moral choices, ethical dilemmas, and untestable ideas. For example, the scientific method cannot tell us if pollution is good or bad. It can tell us the environmental *consequences* of pollution, but whether these consequences are "good" or "bad" is a judgment that we make based on our values or goals, not on science. Although this is an important limitation of the scientific method, science remains one of the most powerful ways of understanding our world.



Figure 1.1 Science is a process of learning about the natural world. Doing experiments that involve gathering repeated and unbiased measurements (data) is at the heart of testing hypotheses and answering questions.

Question 1

What practices besides science are used among world cultures to learn about the natural world?

The questioning and testing inherent in science systematically sift through natural variation to find underlying patterns. The natural world includes much variation, and learning biology would be relatively easy if simple observations accurately revealed patterns of the natural world. But they usually don't—nature is too complicated to rely solely on simple observation. We can certainly learn much about our environment just by looking around us, but casual observations are often biased and misleading because nature varies from time to time and from organism to organism. Biologists need a structured and repeatable process for testing their ideas about the variation in nature. Science is that process.

Question 2
What factors might be responsible for variation in measurements of traits such as the heights of 10-year-old pine trees, or the kidney filtration rates of 10 replicate lab-mice?

The process of science deals with variation primarily through an organized sequence of steps that maintains as much objectivity and repeatability as possible. Although these loosely organized steps, sometimes called the **scientific method,** vary from situation to situation, they are remarkably effective for research and problem solving. The typical steps in the process of science are:

- Make insightful observations
- Pose and clarify testable questions
- Formulate hypotheses
- Do experiments to gather data
- Quantify the data
- Test the hypotheses
- Refine hypotheses and re-test
- Answer the questions and make conclusions

DEVELOPMENT OF OBSERVATIONS, QUESTIONS, AND HYPOTHESES

Make Insightful Observations

Good scientists make insightful observations. But that's not as easy as it seems. Consider these two observations:

Observation 1: There are fewer elk in Yellowstone National Park than there used to be.

Observation 2: The density of elk in Yellowstone National Park has declined during the consecutive dry years since the reintroduction of the native wolf population.

Which of these two observations is the strongest and most useful? Both of them may be true, but the second one is much more insightful because it provides a context to the observation that the elk population is declining. It also suggests a relevant factor—that is, the introduction of the wolf population—as a productive topic for investigation. It also suggests a relationship between density of the elk population and the variation in the local environment.

Procedure 1.1 **Make insightful observations**

1. Consider the following two observations.

Observation 1: Fungi often grow on leftover food.

Observation 2: Fungi such as mold and yeast grow more on leftover bread than on leftover meat.

Which of the above observations is the most useful for further investigation? Why?



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. Briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Record the most insightful of the two observations on Worksheet 1 on page 9.

2. Consider this observation: Pillbugs (sometimes called roly-poly bugs) often find food and shelter where fungi are decomposing leaf litter (fig. 1.2).

For this example we are interested in whether pillbugs are attracted to leaves or to fungi (including yeasts) growing on the leaves' surfaces.

Observation 1: Pillbugs often hide under things.

Propose a more productive observation.

Observation 2: ________________________________

____

Record Observation 2 on Worksheet 2 on page 10. You may revise this later.

Pose and Clarify Testable Questions

Productive observations inspire questions. Humans think in terms of questions rather than abstract hypotheses or numbers. But phrasing a good question takes practice and experience, and the first questions that capture our attention are usually general. For example, "Which nutrients can yeast most readily metabolize?" is a general question that expands the observation posed in procedure 1.1. This question is broadly applicable and is the type of question that we ultimately want to understand. Enter this as the General Question in Worksheet 1.



Figure 1.2 Pillbugs are excellent experimental organisms to test hypotheses about microenvironments, such as those under logs and within leaf litter. Pillbugs are readily available and easily cultured in the lab (10×).

Broad questions are important, but their generality often makes them somewhat vague. The best questions for the process of science are specific enough to answer clearly. Therefore, scientists usually refine and subdivide broad questions into more specific ones. For example, a more specific question is "What classes of biological molecules are most readily absorbed and metabolized by yeast?" Enter this as Specific Question 1 in Worksheet 1.

A further clarification might be "Does yeast absorb and metabolize carbohydrates better than it absorbs and metabolizes proteins?" This is a good, specific question because it clearly refers to organisms, processes, and variables that are likely involved. It also suggests a path for investigation—that is, it suggests an experiment. Enter this as Specific Question 2 in Worksheet 1.

Question 3

Consider the questions "What color is your roommate's car?" and "How many legs do cats have?" To answer these questions, would you use the scientific method, or would you rely on observation? Why?

Procedure 1.2 **Posing and refining questions**

1. Examine the following two questions.

Question 1: Do songbird populations respond to the weather?

Question 2: Do songbirds sing more often in warm weather than in cold weather?

Which of those questions is the most useful for further investigation? Why? ____________________________

____

____

____

2. Examine the following general question, and record it in Worksheet 2.

General Question: What influences the distribution of pillbugs?

Propose a specific question that refers to the food of pillbugs as a variable, and record it here and in Worksheet 2. Know that you may revise this later.

Specific Question 1 ____________________________

____

Propose a more specific question that refers to pillbugs eating leaves, as opposed to pillbugs eating fungi growing on leaves. Record this question here and in Worksheet 2. Know that you may revise this later.

Specific Question 2 ____________________________

____

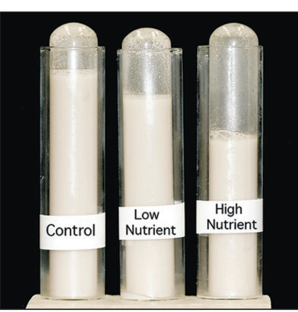


Figure 1.3 These tubes of yeast are fermenting nutrients provided in solution. The CO_2 produced by the yeast accumulates at the top of the test tubes and indicates that yeast's rate of metabolism. From left to right, the tubes include a control with no added nutrients, a tube with low nutrients, and a tube with high nutrients.

Formulate Hypotheses

Well-organized experiments to answer questions require that questions be restated as testable hypotheses. A **hypothesis** is a statement that clearly states the relationship between biological variables. A good hypothesis identifies the organism or process being investigated, identifies the variables being recorded, and implies how the variables will be compared. A hypothesis is a statement rather than a question, and an analysis of your experimental data will ultimately determine whether you accept or reject your hypothesis. Remember that even though a hypothesis can be falsified, it can never be proved true.

Accepting or rejecting a hypothesis, with no middle ground, may seem like a rather coarse way to deal with questions about subtle and varying natural processes. But using controlled experiments to either accept or reject a hypothesis is effective. The heart of science is gathering and analyzing experimental data that leads to rejecting or accepting hypotheses relevant to the questions we want to answer.

In this exercise, you are going to do science as you investigate yeast nutrition and then experiment with food choice by pillbugs. As yeast ferments its food, CO_2 is produced as a by-product. Therefore, we can measure the growth of yeast by measuring the production of CO_2 (fig. 1.3).

A hypothesis related to our question about the growth of yeast might be:

H_0: CO_2 production by yeast fed sugar is not significantly different from the CO_2 production by yeast fed protein.

A related alternative hypothesis can be similarly stated:

H_a: Yeast produces more CO_2 when fed sugar than when fed protein.

The first hypothesis (H_0) is a **null hypothesis** because it states that there is *no difference*. This is the most common way to state a clear and testable hypothesis. (Your instructor may elaborate on why researchers state and test null hypotheses more effectively than alternative hypotheses.) Researchers usually find it more useful to associate statistical probabilities with null hypotheses rather than with alternative hypotheses. Enter the null hypothesis into Worksheet 1.

A well-written null hypothesis is useful because it is testable. In our experiment, the null hypothesis (1) specifies yeast as the organism, population, or group that we want to learn about; (2) identifies CO_2 production as the variable being measured; and (3) leads directly to an experiment to evaluate variables and compare means of replicated measurements.

Procedure 1.3 Formulating hypotheses

1. Examine the following two hypotheses:

   Hypothesis 1: Songbirds sing more when the weather is warm.

   Hypothesis 2: The number of bird songs heard per hour during daylight temperatures above 80°F (27°C) is not significantly different from the number heard per hour at temperatures below 80°F (27°C).

   Which of these hypotheses is the most useful for further investigation? Why?________________________

   ____

   Which of these hypotheses is a null hypothesis? Why?

   ____

2. Examine the following hypothesis.

   Hypothesis 1: Pillbugs prefer leaves coated with a thin layer of yeast.

   Propose a more effective null hypothesis. Be sure that it is a null hypothesis, that it is testable, and that it includes the parameter you will control in an experiment.

   Hypothesis 2 (H_0): ____________________________

   ____

   Enter your null hypothesis in Worksheet 2.

EXPERIMENTATION AND DATA ANALYSIS: YEAST NUTRITION

Gather Experimental Data

To test our hypothesis about yeast growth, we must design a controlled and repeatable experiment. The experiment suggested by our specific question and hypothesis involves offering sugar such as glucose to one population of yeast, offering protein to another population of yeast, and then measuring their respective growth rates. Fortunately, yeast grows readily in test tubes. As yeast grows in a closed, anaerobic container it produces CO_2 in proportion to how readily it uses the available food. CO_2 production is easily measured by determining the volume of CO_2 that accumulates at the top of an inverted test tube.

Experiments provide data that determine if a hypothesis should be accepted or rejected. A well-designed experiment links a biological response to different levels of the variable being investigated. In this case, the biological response is CO_2 production indicating growth. The levels of the variable are sugar and protein. These levels are called **treatments,** and in our experiment they include glucose, protein, and a control. For this experiment the **treatment** (i.e., independent) **variable** being tested is the type of food molecule (i.e., protein, sugar), and the **response** (i.e., dependent) **variable** is the CO_2 production that indicates yeast growth.

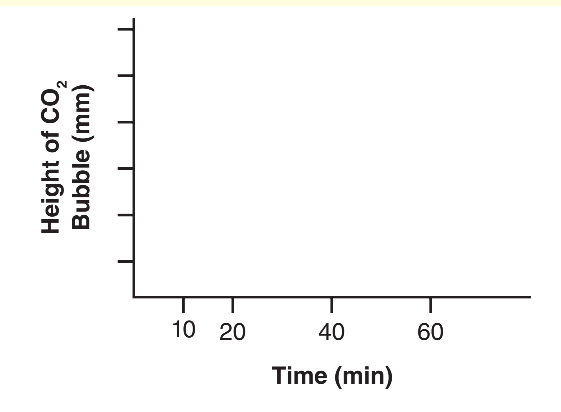
An experiment that compensates for natural variation must be well designed. It should (1) include replications, (2) test only one treatment variable, and (3) include controls.

Replications are repeated measures of each treatment under the same conditions. Replications effectively deal with naturally occurring variation. Usually the more replications, the better. Your first experiment today will include replicate test-tubes of yeast, each being treated the same. Good design tests only one treatment variable at a time.

Good experimental design also requires **controls** to verify that the biological response we measure is a function of the variable being investigated and nothing else. Controls are standards for comparison. They are replicates with all of the conditions of an experimental treatment *except the treatment variable*. For example, if the treatment is glucose dissolved in water, then a control has only water (i.e., it lacks only glucose, the treatment variable). This verifies that the response is to glucose and not to the solvent. Controls validate that our results are due only to the treatment variable.

Procedure 1.4 An experiment to determine the effects of food type on yeast growth

1. Label 12 test tubes as C1–C4, G1–G4, and P1–P4. See Worksheet 1.
2. To test tubes C1–C4 add 5 mL of water. These are control replicates.
3. To test tubes G1–G4 add 5 mL of 5% glucose solution. These are replicates of the glucose treatment.
4. To test tubes P1–P4 add 5 mL of 5% protein solution. These are replicates of the protein treatment.
5. Swirl the suspension of yeast until the yeast is distributed uniformly in the liquid. Then completely fill the remaining volume in each tube with the yeast suspension that is provided.
6. For each tube, slide an inverted, flat-bottomed test tube down over the yeast-filled tube. Hold the yeast-filled tube firmly against the inside bottom of the cover tube and invert the assembly. Your instructor will demonstrate how to slip this slightly larger empty tube over the top of each yeast tube and invert the assembly. If done properly, no bubble of air will be trapped at the top of the tube of yeast after inversion.
7. Place the tubes in a rack and incubate them at 50°C.
8. Measure the height (mm) of the bubble of accumulated CO_2 after 10, 20, 40, and 60 minutes. Record your results in Worksheet 1 and graph them here:



9. When you are finished, clean your work area and dispose of the contents of each tube as instructed by your lab instructor.

Test Your Predictions by Analyzing the Experimental Data

Analysis begins with summarizing the raw data for biological responses to each treatment. The first calculation is the **mean** $(\bar{x})$ of the response variable for replicates of each treatment, and for the control replicates. The mean is a single number that represents the central tendency of the response variable. Later the mean of each treatment will be compared to determine if the treatments had different effects.

The second step in data analysis is to calculate variation within each set of replicates. The simplest measure of variation is the **range,** which is the highest and lowest values in a set of replicates. A wide range indicates much variation in the data. The **standard deviation (SD),** another informative measure of variation, summarizes variation just as the range does, but the standard deviation is less affected by extreme values. Refer to the box "Variation in Replicate Measures" to learn how to calculate the standard deviation.

Question 4

Even the seemingly simple question "How tall are mature males of the human species?" can be difficult to answer. How would you best express the answer?

Procedure 1.5 Quantify and summarize the data

1. Examine your raw data in Worksheet 1.
2. Calculate the mean of the response variable (CO_2 production) for the four control replicates. To calculate the means for the four replicates, sum the four values and divide by four. Record the mean for the control replicates in Worksheet 1.
3. The CO_2 production for each glucose and protein replicate must be adjusted with the control mean. This ensures that the final data reflect the effects of only the treatment variable and not the solvent. Subtract the control mean from the CO_2 production of each glucose replicate and each protein replicate, and record the results in Worksheet 1.
4. Record in Worksheet 1 the range of adjusted CO_2 production for the four replicates of the glucose treatment and of the protein treatment.
5. Calculate the mean CO_2 production for the four adjusted glucose treatment replicates. Record the mean in Worksheet 1.
6. Calculate the mean CO_2 production for the four adjusted protein treatment replicates. Record the mean in Worksheet 1.
7. Refer to "Variation in Replicate Measures," and calculate the standard deviation for the four adjusted glucose treatment values and for the four adjusted protein treatment values. Record the two standard deviations in Worksheet 1.

Variation in Replicate Measures

Natural variation occurs in all processes of biology. This variation will inevitably produce different results in replicated treatments. One of the most useful measures of variation of values about the mean is **standard deviation.** It's easy to calculate: calculate the mean, calculate the deviation of each sample from the mean, square each deviation, and then sum the deviations. This summation is the sum of squared deviations. For example, data for CO_2 production by yeast in four replicate test tubes might be 22, 19, 18, and 21 mm. The mean is 20 mm.

CO_2 Production (mm)	Mean	Deviation	Deviation^2
22	20	2	4
19	20	−1	1
18	20	−2	4
21	20	1	1
	Sum of squared deviations =		10

The summary equation for the sum of squared deviations is

$$\textbf{Sum of squared deviations} = \sum_{i=1}^{N}(x_i - \bar{x})^2$$

where

N = **total number of samples**

$\bar{x}$ = **the sample mean**

x_i = **measurement of an individual sample**

The summation sign ($\sum_{i=1}^{N}$) means to add up all the squared deviations from the first one ($i = 1$) to the last one ($i = N$).

The sum of squared deviations (10) divided by the number of samples minus one (4 − 1 = 3) produces a value of 10/3 = 3.3 mm^2 (the units are millimeters squared). This is the variance:

$$\textbf{Variance} = \frac{\textbf{sum of squared deviations}}{N - 1}$$

The square root of the variance, 1.8 cm, equals the standard deviation

$$\textbf{SD} = \sqrt{\textbf{Variance}} = \sqrt{3.3} = 1.8$$

The standard deviation is often reported with the mean in statements such as, "The mean CO_2 production was 20 ± 1.8 mm." The standard deviation helps us understand the spread or variation among replicated treatments.

Test the Hypotheses

Our hypothesis about yeast growth is tested by comparing the mean CO_2 production by yeast fed glucose to the mean CO_2 production by yeast fed protein. However, only determining if one mean is higher than the other is not an adequate test because natural variation will always make the two means at least slightly different, even if the two treatments have the same effect on yeast growth. Therefore, the means and the variation about the means must be compared to determine if the means are not just different but **significantly different.** To be significantly different, the differences between means must be due to the treatment and not just due to natural variation. If the difference is significant, then the null hypothesis is rejected. If the difference is not significant, then the null hypothesis is accepted. Testing for significant differences is usually done with statistical methods.

Statistical methods calculate the probability that the means are significantly different. But these complex calculations are beyond the scope of this exercise. We will use a simpler method to test for a significant difference between the means of our two treatments. We will declare that two means are significantly different *if the means plus or minus 1/2 of the standard deviation do not overlap*.

For example, consider these two means and their standard deviations (SD):

$Mean_a = 10$ SD = 5	$Mean_b = 20$ SD = 10
$Mean_a - (½)SD = 7.5$	$Mean_b - (½)SD = 15$
$Mean_a + (½)SD = 12.5$	$Mean_b + (½)SD = 25$

Are $Mean_a$ and $Mean_b$ significantly different according to our test for significance? Yes they are, because 7.5 ↔ 12.5 does not overlap 15 ↔ 25.

Procedure 1.6 **Testing hypotheses**

1. Consider your null hypothesis and the data presented in Worksheet 1.
2. Calculate the glucose mean − (½)SD and the glucose mean + (½)SD. Record them in Worksheet 1.
3. Calculate the protein mean − (½)SD and the protein mean + (½)SD. Record them in Worksheet 1.
4. Do the half standard-deviations surrounding the means of the two treatments overlap? Record your answer in Worksheet 1.
5. Are the means for the two treatments significantly different? Record your answer in Worksheet 1.
6. Is your null hypothesis accepted? Or rejected? Record your answer in Worksheet 1.

Answer the Questions

The results of testing the hypotheses are informative, but it still takes a biologist with good logic to translate these results into the answers of our specific and general questions. If your specific questions were well stated, then answering them based on the results of your experiment and hypothesis testing should be straightforward.

Procedure 1.7 **Answering the questions: yeast nutrition**

1. Examine the results of hypothesis testing presented in Worksheet 1.
2. Specific Question 2 was "Does yeast absorb and metabolize carbohydrates better than it absorbs and metabolizes proteins?" Enter your answer in Worksheet 1.
3. Does your experiment adequately answer this question? Why or why not?

4. Specific Question 1 was "What classes of biological molecules are most readily absorbed and metabolized by yeast?" Enter your best response in Worksheet 1.
5. Does your experiment adequately answer Specific Question 1? Why or why not?

6. The General Question was "Which nutrients can yeast most readily metabolize?" After testing the hypotheses, are you now prepared to answer this general question? Why or why not?

EXPERIMENTATION AND DATA ANALYSIS: FOOD PREFERENCE BY PILLBUGS

In the previous procedures you developed and recorded observations, questions, and hypotheses concerning food preference by pillbugs. Pillbugs may be attracted to dead leaves as food, or they may be attracted to fungi growing on the leaves as food. Leaves dipped in a yeast suspension can simulate fungi growing on leaves. Use the following procedures as a guide to the science of experimentation and data analysis to test the hypothesis you recorded in Worksheet 2.

Procedure 1.8 **Design an experiment to test food preference by pillbugs**

1. Design an experiment to test your hypothesis in Worksheet 2 about food preference by pillbugs. To do this, specify:

   Experimental setup ______________________________

   ____

   Treatment 1 to be tested __________________________

   ____

Treatment 2 to be tested ________________________

____

Control treatment ________________________

____

Response variable ________________________

____

Treatment variable ________________________

____

Number of replicates ________________________

____

Means to be compared ________________________

____

2. Conduct your experiment and record the data in Worksheet 2.
3. Analyze your data. Record the control means and adjusted treatment-means in Worksheet 2.
4. Calculate the range and standard deviation for your treatments, and record them in Worksheet 2.
5. Test your hypothesis. Determine if the null hypothesis should be accepted or rejected. Record the results in Worksheet 2.
6. Answer the Specific Question 2, Specific Question 1, and the General Question posed in Worksheet 2.

Procedure 1.9 Answering the questions: food preference by pillbugs

1. Examine the results of your hypothesis testing presented in Worksheet 2.
2. Enter your answer to Specific Question 2 in Worksheet 2. Does your experiment adequately answer this question? Why or why not?
3. Enter your best response to Specific Question 1 in Worksheet 2. Does your experiment adequately answer this question? Why or why not?
4. After testing the hypotheses, are you now prepared to answer your General Question "What influences the distribution of pillbugs?" Why or why not?

Question 5
What are some examples of biological theories?

Scientific Theories

Throughout this course you will make many predictions and observations about biology. When you account for a group of these observations with a generalized explanation, you have proposed a scientific theory.

In science, as opposed to common usage, a theory is a well-substantiated explanation of some aspect of the natural world that usually incorporates many confirmed observational and experimental facts. A scientific theory makes predictions consistent with what we see. It is not a guess; on the contrary, a scientific theory is widely accepted within the scientific community—for example, the germ theory claims that certain infectious diseases are caused by microorganisms. Scientific theories do not become facts; scientific theories *explain* facts.

INVESTIGATION

How Temperature Affects the Production of CO_2 by Yeast

Observation: Fermentation of nutrients by yeast produces CO_2, and the production rate of this CO_2 can be used to measure growth of the yeast. In this lab you've already investigated how the production of CO_2 is affected by different nutrients (i.e., sugar, protein). Here you'll investigate another variable: temperature.

Question: How is the production of CO_2 by yeast affected by temperature?

a. Establish a working lab group and obtain Investigation Worksheet 1 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis. Record this hypothesis.
d. Outline on Worksheet 1 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. Newspaper articles often refer to a discovery as "scientific" or claim that something has been proved "scientifically." What is meant by this description?

2. Experimental results in science are usually reviewed by other scientists before they are published. Why is this done?

3. Have all of our discoveries and understandings about the natural world been the result of applying the scientific method? How so?

4. Suppose that you hear that two means are *significantly* different. What does this mean?

5. Can means be different but not significantly different? Explain your answer.

6. How can science be used to address "big" issues such as climate change?

7. Some people dismiss evolution by natural selection as being "only a theory." Biologists often respond that yes, evolution *is* a scientific theory. What does this mean?

8. A hallmark of a scientific theory is that it is falsifiable. What does this mean, and why is it important?

Worksheet 1 The Process of Science: Nutrient Use in Yeast

OBSERVATION ____

QUESTIONS

General Question: ____

Specific Question 1: ____

Specific Question 2: ____

HYPOTHESIS H_0: ____

EXPERIMENTAL DATA: Nutrient Use in Yeast

Treatments — Treatments Minus Control $\bar{x}$

Replicate	Control CO_2 Production (mm)	Replicate	Glucose CO_2 Production (mm)	Replicate	Protein CO_2 Production (mm)	Glucose CO_2 Production Adjusted for the Control $\bar{x}$	Protein CO_2 Production Adjusted for the Control $\bar{x}$
C1	______	G1	______	P1	______	______	______
C2	______	G2	______	P2	______	______	______
C3	______	G3	______	P3	______	______	______
C4	______	G4	______	P4	______	______	______

Control $\bar{x}$ = ______

Glucose $\bar{x}$ = ______

Glucose range = ______ – ______

Glucose SD = ______

Protein $\bar{x}$ = ______

Protein range = ______ – ______

Protein SD = ______

TEST HYPOTHESIS

Glucose $\bar{x} - (½)SD$ = ______

Glucose $\bar{x} + (½)SD$ = ______

Protein $\bar{x} - (½)SD$ = ______

Protein $\bar{x} + (½)SD$ = ______

Do the half standard deviations surrounding the means of the two treatments overlap? Yes ______ No ______

Are the means for the two treatments significantly different? Yes ______ No ______

Is the null hypothesis accepted? ______ or rejected? ______

ANSWER QUESTIONS

Answer to Specific Question 2 ____

Answer to Specific Question 1 ____

Answer to General Question ____

Worksheet 2 The Process of Science: Food Preference by Pillbugs

OBSERVATION ____

QUESTIONS

General Question: ____

Specific Question 1: ____

Specific Question 2: ____

HYPOTHESIS H_0: ____

EXPERIMENTAL DATA: Food Preference by Pillbugs

Treatments						Treatments Minus Control $\bar{x}$	
Replicate	**Control**	**Replicate**	**Treatment 1**	**Replicate**	**Treatment 2**	**Treatment 1 Adjusted for the Control $\bar{x}$**	**Treatment 2 Adjusted for the Control $\bar{x}$**
1	______	1	______	1	______	______	______
2	______	2	______	2	______	______	______
3	______	3	______	3	______	______	______
4	______	4	______	4	______	______	______

Control $\bar{x}$ = ______

Treatment 1 $\bar{x}$ = ______

Treatment 1 range = ______ – ______

Treatment 1 SD = ______

Treatment 2 $\bar{x}$ = ______

Treatment 2 range = ______ – ______

Treatment 2 SD = ______

TEST HYPOTHESIS

Treatment 1 $\bar{x}$ – (½)SD = ______

Treatment 1 $\bar{x}$ + (½)SD = ______

Treatment 2 $\bar{x}$ – (½)SD = ______

Treatment 2 $\bar{x}$ + (½)SD = ______

Do the half standard deviations surrounding the means of the two treatments overlap? Yes ______ No ______

Are the means for the two treatments significantly different? Yes ______ No ______

Is the null hypothesis accepted? ______ or rejected? ______

ANSWER QUESTIONS

Answer to Specific Question 2 ____

Answer to Specific Question 1 ____

Answer to General Question ____



Exercise 2

Measurements in Biology

The Metric System and Data Analysis

Learning Objectives

By the end of this exercise you should be able to:

1. Understand the difference between accuracy and precision in measurements.
2. Identify the metric units used to measure length, volume, mass, and temperature.
3. Measure length, volume, mass, and temperature in metric units.
4. Convert one metric unit to another (e.g., grams to kilograms).
5. Use measures of volume and mass to calculate density.
6. Practice the use of simple statistical calculations such as mean, median, range, and standard deviation.
7. Analyze sample data using statistical tools.



Please visit connect.mheducation.com to review online resources tailored to this lab.

Every day we're bombarded with numbers and measurements. They come at us from all directions, including while we're at the supermarket, gas station, golf course, and pharmacy, as well as while we're in our classrooms and kitchens. Virtually every package that we touch is described by a measurement.

Scientists use a standard method to collect data as well as use mathematics to analyze measurements. We must measure things before we can objectively describe what we are observing, before we can experiment with biological processes, and before we can predict how organisms respond, adjust to, and modify their world. Once we have made our measurements, we can analyze our data and look for variation and the sources of that variation. Then we can infer the causes and effects of the biological processes that interest us.

ACCURACY AND PRECISION

Scientists strive to make accurate, precise measurements. The **accuracy** of a group of measurements refers to how closely the measured values agree with the true or correct value. In contrast, the **precision** of a group of measurements refers to how closely the measurements agree with each other. That is, precision is the degree to which the measurements produce the same results, regardless of their accuracy. Measurements that are both accurate and precise are **valid** measurements. Scientists strive to make valid measurements.

Question 1

a. Can measurements be accurate but not precise? Explain.

b. Can measurements be precise but not accurate? Explain.

To help you check your answers, consider an analogy involving shooting arrows at a bull's-eye target (fig. 2.1). In this

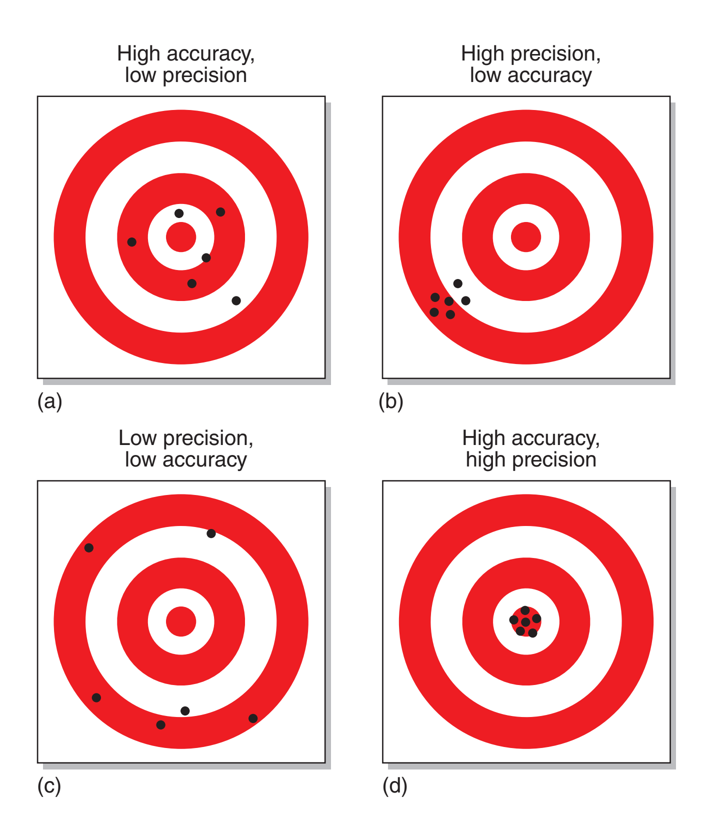


Figure 2.1 Precision and accuracy. Measurements can be (*a*) accurate but not precise, (*b*) precise but not accurate, (*c*) neither precise nor accurate, or (*d*) both precise and accurate. Measurements that are precise and accurate are termed *valid*.

analogy, each arrow would represent a measurement. Accuracy would be the closeness of the arrows to the center of the target; arrows closest to the bull's-eye would be most accurate. Precision would be the size of the cluster of arrows, regardless of how close they are to the center of the target.

THE METRIC SYSTEM

Scientists throughout the world use the **metric system** to make measurements. The metric system is also used in everyday life virtually everywhere except the United States. With few exceptions (e.g., liter bottles of soda), most measurements in the United States use the antiquated English system of pounds, inches, feet, and so on. Check with your instructor about bringing to class common grocery store items with volumes and weights in metric units, or examining those items on display.

Metric measurement is used worldwide in science to improve communication in the scientific community. Scientists make all of their measurements in the metric system; they do not routinely convert from one system to another. When scientists have mixed metric units with English units, the results have often been confusing, and have sometimes been disastrous. For example, in 1999, the $125-million Mars Climate Orbiter was approaching Mars to study the planet's climate. Lockheed Martin Astronautics, which built the spacecraft, gave NASA critical flight information in English units, but software aboard the orbiter expected the data in metric units. As a result, the orbiter was sent into, rather than safely above, the Mars atmosphere, where it disintegrated.

The following conversions will help give you a sense of how some common English units are related to their metric equivalents:

1 inch = 2.5 centimeters
1 foot = 30 centimeters
1 yard = 0.9 meter
1 mile = 1.6 kilometers
1 ounce = 28 grams
1 pound = 0.45 kilogram
1 fluid ounce = 30 milliliters
1 pint = 0.47 liter
1 quart = 0.95 liter
1 gallon = 3.8 liters
1 cup = 0.24 liter

If you want to know more about these conversions, see Appendix II.

This exercise will introduce you to making metric measurements of length, mass, volume, and temperature. During this lab, you should spend your time making measurements, not reading background information. Therefore, *before lab*, *read this exercise carefully to familiarize yourself with the basic units of the metric system.*

Metric units commonly used in biology include:

meter (m)—the basic unit of length
liter (L)—the basic unit of volume
kilogram (kg)—the basic unit of mass
degrees Celsius (°C)—the basic unit of temperature

Unlike the English system with which you are already familiar, the metric system is based on units of ten. This simplifies conversions from one metric unit to another (e.g., from kilometers to meters). This base-ten system is similar to our monetary system, in which 10 cents equals a dime, 10 dimes equals a dollar, and so forth. Units of ten in the metric system are indicated by Latin and Greek prefixes placed before the base units:

Prefix (Latin)		*Division of Metric Unit*	
deci	(d)	0.1	10^{-1}
centi	(c)	0.01	10^{-2}
milli	(m)	0.001	10^{-3}
micro	(μ)	0.000001	10^{-6}
nano	(n)	0.000000001	10^{-9}
pico	(p)	0.000000000001	10^{-12}

Prefix (Greek)		*Multiple of Metric Unit*	
deka	(da)	10	10^{1}
hecto	(h)	100	10^{2}
kilo	(k)	1000	10^{3}
mega	(M)	1000000	10^{6}
giga	(G)	1000000000	10^{9}

Thus, multiply by

0.01 to convert centimeters to meters
0.001 to convert millimeters to meters
1000 to convert kilometers to meters
0.1 to convert millimeters to centimeters

For example, there are 10 millimeters per centimeter. Therefore, to convert 62 centimeters to millimeters,

$$62 \text{ cm} \times \frac{10 \text{ mm}}{\text{cm}} = 620 \text{ mm}$$

In these conversion equations, the units being converted *from* (in this case, centimeters) cancel out, leaving you with the desired units (in this case, millimeters). Also note that when units are converted to *smaller* units, the number associated with the new units will *increase*, and vice versa. For example, 620 meters = 0.620 kilometers = 620,000 millimeters = 62,000 centimeters.

Question 2

Make the following metric conversions:

1 meter =______centimeters =______millimeters

92.4 millimeters =______meters =______centimeters

10 kilometers =______meters =______decimeters

82 centimeters =______meters =______millimeters

3.1 kilograms =______grams =______milligrams

281 milliliters =______liters =_______deciliters

35 millimeters =_____centimeters =_______meters

Length and Area

The **meter** (m) is the basic unit of length. Units of area are squared units (i.e., two-dimensional) of length.

1 m = 100 cm = 1000 mm = 0.001 km =1×10^{-3} km
1 km = 1000 m = 10^3 m
1 cm = 0.01 m = 10^{-2} m = 10 mm
470 m = 0.470 km
1 cm^2 = 100 mm^2 (i.e., 10 mm × 10 mm = 100 mm^2)

To help you appreciate the magnitudes of these units, here are the lengths and areas of some familiar objects:

Length	
Housefly	0.5 cm
Diameter of penny	1.9 cm
Diameter of baseball	7.4 cm
Soda can	12.2 cm
Toyota Camry	4.7 m
Mt. Everest	8848 m

Area	
Credit card	46 cm^2
Total skin area of adult human male	1.8 m^2
Ping-pong table	4.18 m^2
Surface area of human lungs	80 m^2
Football field (goal line to goal line)	4459 m^2
Central Park (New York City)	3.4 km^2

Procedure 2.1 **Make metric measurements of length and area**

Most biologists measure lengths with metric rulers or metersticks.

1. Examine intervals marked on the metric rulers and metersticks available in the lab.
2. Make the following measurements. Be sure to include units for each measurement.

   Length of this page __________
   Width of this page __________
   Area of this page (Area = Length × Width) __________
   Your height __________
   Thickness of this manual __________
   Height of a 200-mL beaker __________
   Height of ceiling __________
   Height of your chair __________
   Length of your cell phone __________

Question 3
What are some potential sources of error in your measurements?

Volume

Volume is the space occupied by an object. Units of volume are cubed (i.e., three-dimensional) units of length. The liter (L) is the basic unit of volume.

1 L = 1000 cm^3 = 1000 mL
1 L = 0.1 m × 0.1 m × 0.1 m
1 cm^3 = 0.000001 m^3

To help you appreciate the magnitudes of these units, here are the volumes of some familiar objects:

Chicken egg	60 mL
Coke can	355 mL
One breath of air	500 cm^3

Scientists often measure volumes with pipets and graduated cylinders. Pipets are used to measure small volumes, typically 25 mL or less. Liquid is drawn into a pipet using a bulb or pipet pump (fig. 2.2). Never pipet by mouth.

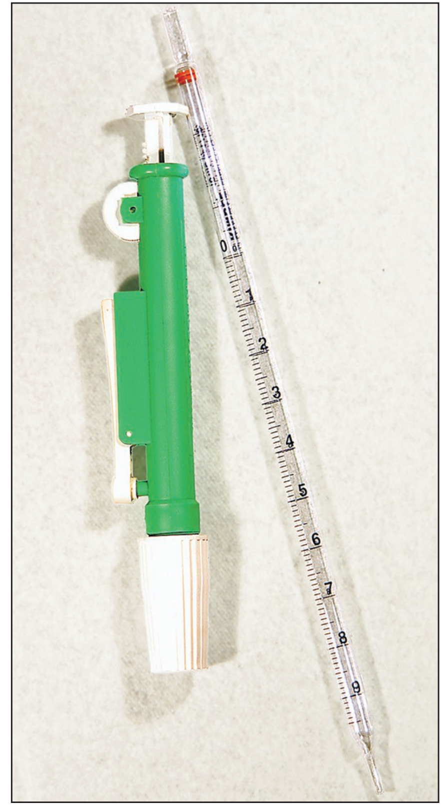
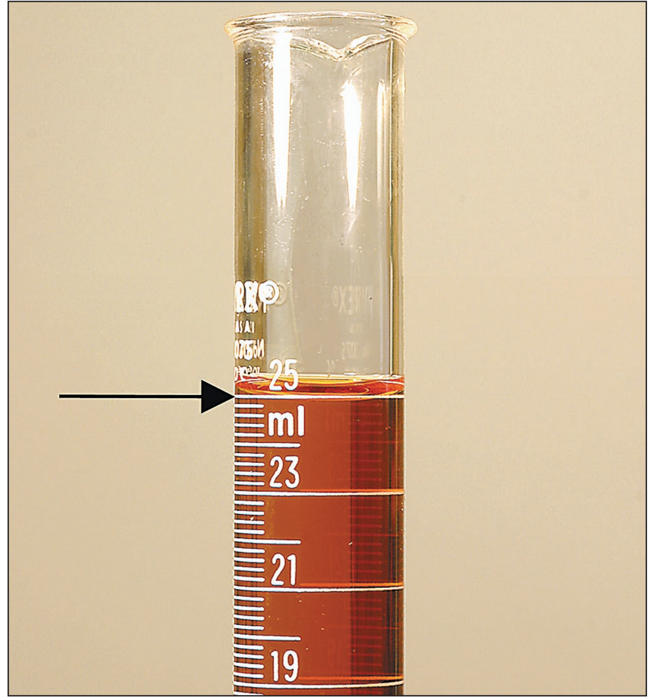


Figure 2.2 A pipet is used to extract and dispense volumes of liquid. A suction device (shown in green on the left) draws fluid into the pipet, and graduated markings on the pipet allow precise measurement of a fluid's volume. Never use your mouth to suck fluid into a pipet.



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Figure 2.3 When measuring the volume of liquid in a graduated cylinder, always measure at the bottom of the meniscus. The bottom of the meniscus in this photograph is indicated by the arrow. The correct volume is 25 mL.

Graduated cylinders are used to measure larger volumes. To appreciate how to make a measurement accurately, pour 40–50 mL of water into a 100-mL graduated cylinder, and observe the interface between the water and air. This interface, called the **meniscus,** is curved because of surface tension and the adhesion of water to the sides of the cylinder. When measuring the liquid in a cylinder such as a graduated cylinder, always position your eyes level with the meniscus and read the volume at the lowest level (fig. 2.3).

Procedure 2.2 Make metric measurements of volume

1. Biologists often use graduated cylinders to measure volumes. Locate the graduated cylinders available in the lab to make the following measurements. Determine what measurements the markings on the graduated cylinder represent. Be sure to include units for each measurement.
2. Measure the milliliters needed to fill a cup (provided in the lab). ________________
3. Measure the liters in a gallon. _______________

Procedure 2.3 Measure the volume of a solid object by water displacement

1. Obtain a 100-mL graduated cylinder, a thumb-sized rock, and a glass marble.
2. Fill the graduated cylinder with 70 mL of water.
3. Gently submerge the rock in the graduated cylinder. Notice that the volume of the contents rises.
4. Carefully observe the meniscus of the fluid and record its volume.
5. Calculate and record the volume of the rock by subtracting the original volume (70 mL) from the new volume.

   Rock volume ____________
6. Repeat steps 2–5 to measure and record the volume of the marble.

   Marble volume ____________

Biologists use pipets to measure and transfer small volumes of liquid from one container to another. The following procedure will help you appreciate the usefulness of pipets.

Procedure 2.4 Learn to use a pipet

1. Add approximately 100 mL of water to a 100-mL beaker.
2. Use a 5-mL pipet with a bulb or another filling device provided by your instructor to remove some water from the beaker.
3. Fill the pipet to the zero mark.
4. To read the liquid level correctly, your eye must be directly in line with the bottom of the meniscus.
5. Release the liquid into another container.

Question 4

What volume of liquid did you measure?

Mass

The **kilogram** (kg) is the basic unit of mass.[1] A kilogram equals the mass of 1000 cubic centimeters (cm^3) of water at 4°C. Similarly,

$$\mathbf{1\ kg = 1000\ g = 10^3\ g \qquad 1\ mg = 0.001\ g = 10^{-3}\ g}$$

Here are the masses of some familiar objects:

Housefly	12 mg	9V battery	40 g
Hummingbird	1.6 g	Human heart	300 g
Ping-pong ball	2.45 g	Basketball	0.62 kg
Quarter	6.25 g		

Biologists usually measure mass with a top-loading balance or a triple-beam balance (fig. 2.4). Locate the triple-beam balances or top-loading electronic balances in the lab. Triple-beam balances get their names from their three

1 *Remember that mass is not necessarily synonymous with weight. Mass measures an object's* **potential** *to interact with gravity, whereas weight is the force exerted by gravity on an object. Thus, a weightless object in outer space has the same mass as it has on earth.*

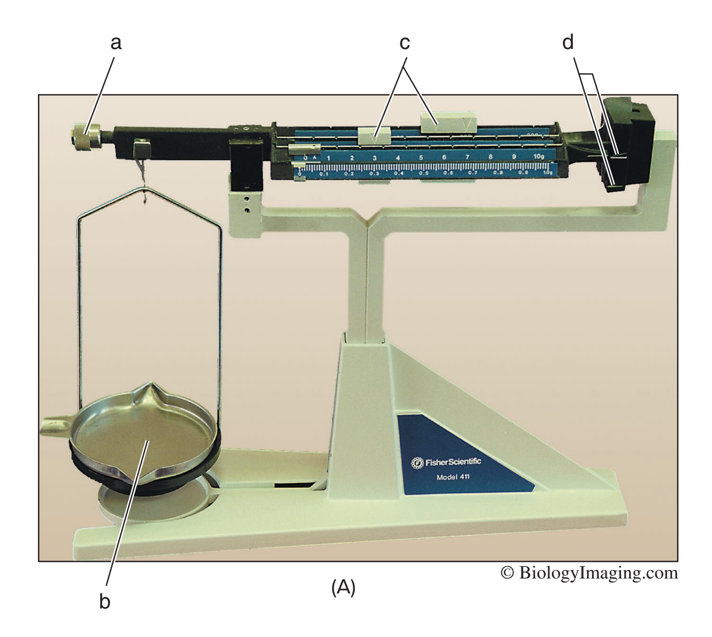




Figure 2.4 Biologists use balances to measure mass. (A) The parts of a triple-beam balance include the (*a*) zero-adjustment knob, (*b*) measuring pan, (*c*) movable masses on horizontal beams, and (*d*) balance marks. (B) A top-loading balance has a measuring pan, a power switch, and a zero calibration ("Tare") button.

horizontal beams. Suspended from each of the three beams are movable masses. Each of the three beams of the balance is marked with graduations: the closest beam has 0.1-g graduations, the middle beam has 100-g graduations, and the farthest beam has 10-g graduations.

Procedure 2.5 Make metric measurements of mass

1. Before making any measurements, clean the weighing pan and move all of the suspended weights to the far left. The balance marks should line up to indicate zero grams; if they do not, turn the adjustment knob until they do. Measure the mass of an object by placing it in the center of the weighing pan and moving the suspended masses until the beams balance. The mass of the object is the sum of the masses indicated by the weights on the three beams.
2. If you're using an electronic balance, turn on the balance and let it warm up for 5 minutes. Wait until the display reads 0.0 g; if the display does not read 0.0 g, press the "Tare" button to reset the display to 0.0 g. If you are weighing an object such as a coin or pencil, place the object on the measuring pan. After the display has stabilized, read and record the object's mass.
3. If you are weighing a liquid, powder, or similar specimen, place an empty beaker (in which you will place the liquid) or a piece of weighing paper (on which you will place the powder) on the balance's measuring pan. After the display has stabilized, press the "Tare" button to reset the display to 0.0 g. Place the liquid in the beaker (or the powder on the weighing paper). After the display has stabilized, read and record the mass.
4. Measure the masses of the following items. Be sure to include units for each measurement.

   Penny _______________

   Paper clip _______________

   Pencil _______________

   Rock (used in procedure 2.3) _____________

   100-mL beaker (empty) _____________

   100-mL beaker containing 50 mL of water _________

Question 5

a. **Density** is mass per unit volume. Use data that you've gathered to determine the density of water at room temperature.

Density of water = (mass/volume) = __________

b. What is the density of the wooden pencil? Does it float? Why?

c. What is the density of the rock? Does it sink? Why?

Temperature

Temperature is the measure of the kinetic energy of molecules—that is, the amount of heat in a system. Biologists measure temperature with a thermometer calibrated in degrees Celsius (°C). The Celsius scale is based on water freezing at 0°C and boiling at 100°C. You can interconvert °C and degrees Fahrenheit (°F) by using the formula 5(°F) = 9(°C) + 160. Here are some typical temperatures:

−20°C	temperature in a freezer
−18°C	mixture of ice and salt
0°C	water freezes
4°C	temperature in a refrigerator
22°C	room temperature
30.6°C	butter melts
37°C	human body temperature
40°C	a hot summer day
50°C	hottest day on record in Phoenix, AZ
71°C	flash pasteurization of milk
75°C	hot coffee
100°C	water boils
260°C	broiler temperature

Procedure 2.6 **Make metric measurements of temperature**

1. Obtain a thermometer in the lab. Handle the thermometer with care. If it breaks, notify your instructor immediately.

2. Determine the range of the temperatures that can be measured with your thermometer by examining the scale imprinted along the barrel of the thermometer.

3. Measure the following temperatures:

Room temperature	______________	°C
Cold tap water	______________	°C
Hot tap water	______________	°C
Inside refrigerator	______________	°C

UNDERSTANDING NUMERICAL DATA

Statistics offer a way to organize, summarize, and describe data—the data are usually samples of information from a much larger population of values. Statistics and statistical tests allow us to analyze the sample and draw inferences about the entire population. Consequently, the use of statistics enables us to make decisions even though we have incomplete data about a population. Although this may seem unscientific, we do it all the time; for example, we diagnose diseases with a drop of blood. Decisions are based on statistics when it is impossible or unrealistic to analyze an entire population.

Let's say that you want to know the mass of a typical apple in your orchard. To obtain this information, you could analyze one apple, but how would you know that you'd picked a "typical" sample? After all, the batch from

Significant Figures

Let's suppose that you're measuring the length of a bone, as shown in figure 2.5. How would you record this length—as 8 cm? 8.3 cm? 8.33 cm? 8.33333 cm? To answer this question, you need to know something about significant figures.

Significant figures are the number of figures required to record a measurement so that only the last digit in the number is in doubt. For example, if the ruler you're using is calibrated only in centimeters and you find that the object you're measuring is between 8 and 9 cm long (fig. 2.5), then you should estimate your measurement only to a tenth of a centimeter. That is, a measurement of 8.3 cm is acceptable, but 8.33 is not because it implies a precision that did not exist in the equipment you used to make the measurement. If, however, your ruler was calibrated in millimeters, then 8.33 cm would be acceptable. Remember this: When recording measurements, include all of the digits you are sure of plus an estimate to the nearest one-tenth of the next smaller digit.

Here are some other guidelines for using the correct number of significant figures in your measurements:

When adding or subtracting measurements, the answer should have no more precision than the measurement having the least number of significant figures. For example, suppose the air temperature in an incubator drops from 8.663°C to 8.2°C. This is a difference of 8.663°C – 8.2°C = 0.5°C, not 0.463°C. If the second temperature reading had been 8.200°C, then the correct answer would have been 0.463°C.

When converting measurements from one set of units to another, do not introduce precision that is not present in the first number. For example, 8.3 cm = 83 mm, not 83.0 mm.

When manipulating two measurements simultaneously, the precision of the final measurement should not exceed that of the least number of significant figures. For example, the calculation for the mass of 17.2 mL of water is 17.2 mL × 0.997821 g mL^{-1} = 17.2 g, not 17.162521 g.

Figure 2.5 How long is this bone? 8 cm? 8.3 cm? 8.33 cm?

which you chose the apple may contain many others, each a little different. You'd get a better estimate of "typical" if you increased your sample size to a few hundred apples, or even to 10,000. Or, better yet, to 1,000,000.

The only way to be certain of your conclusions would be to accurately measure all the apples in your orchard. This is impossible, so you must choose apples that *represent* all of the other apples—that is, you must be working with a *representative sample*. A statistical analysis of those sample apples reduces the sample values to a few characteristic measurements (e.g., mean mass). As you increase the size of the sample, these characteristic measurements provide an ever-improving estimation of what is "typical."

There are a variety of software programs that perform statistical analyses of data; all you have to do is enter your data into a spreadsheet, select the data that you want to analyze, and perform the analysis. Although these software packages save time and can increase accuracy, you still need to understand a few of the basic variables that you'll use to understand your numerical data. We'll start with the mean and median:

The **mean** is the arithmetic average of a group of measurements. Chance errors in measurements tend to cancel themselves when means are calculated for relatively large samples; a value that is too high because of random error is often balanced by a value that is too low for the same reason.

The **median** is the middle value of a group of measurements.

The median is less sensitive to extreme values than is the mean. To appreciate this, consider a sample consisting of 14 leaves having the following lengths (all in mm):

80 69 62 74 69 51 45 40 9 64 65 64 61 67

The mean length is 58.6 mm. However, none of the leaves are that length, and most of the leaves are longer than 60 mm. In biology, the mean is usually preferred to the median when reporting descriptive statistics.

Question 6

a. Does the mean always describe the "typical" measurement? Why or why not?

b. What information about a sample does a mean *not* provide?

Determine the median by arranging the measurements in numerical order:

9 40 45 51 61 63 64 64 65 67 69 69 73 80

The median is between the seventh and eighth measurement: 64 mm. In this sample, the mean differs from the median.

Question 7

a. What is responsible for this difference between the mean and median?

b. How would the median change if the 9-mm-long leaf was not in the sample?

c. How would the mean change if the 9-mm-long leaf was not in the sample?

d. Consider these samples:

Sample 1: 25 35 32 28

Sample 2: 15 75 10 20

What is the mean for Sample 1? ____________

What is the mean for Sample 2? ____________

In most of the exercises in this manual, you'll have time to make only one or two measurements of a biological structure or phenomenon. In these instances, a mean may be the only descriptor of the sample. However, if your class combines its data so that there are many measurements, you'll

Hints for Using the Metric System

1. Use decimals, not fractions (e.g., 2.5 m, not $2\frac{1}{2}$ m).
2. Express measurements in units requiring only a few decimal places. For example, 0.3 m is more easily manipulated and understood than 300000000 nm.
3. When measuring pure water, the metric system offers an easy and common conversion from volume measured in liters to volume measured in cubic meters to mass measured in grams: 1 mL = 1 cm^3 = 1 g.
4. The metric system uses symbols rather than abbreviations. Therefore, do not place a period after metric symbols (e.g., 1 g, not 1 g.). Use a period after a symbol only at the end of a sentence.
5. Do not mix units or symbols (e.g., 9.2 m, not 9 m 200 mm).
6. Metric symbols are always singular (e.g., 10 km, not 10 kms).
7. Except for degrees Celsius, always leave a space between a number and a metric symbol (e.g., 20 mm, not 20mm; 10°C, not 10° C).
8. Use a zero before a decimal point when the number is less than one (e.g., 0.42 m, not .42 m).

need to know how to do a couple of other calculations so that you understand the variation within your sample.

Variability

As you can see, the samples in Question 7d are different, but their means are the same. Thus, the mean does not reveal all there is to know about these samples. To understand how these samples are different, you need other statistics: the range and standard deviation.

The **range** is the difference between the extreme measurements (i.e., smallest and largest) of the sample. In Sample 1, the range is 35 − 25 = 10; in Sample 2 the range is 75 − 10 = 65. The range provides a sense of the variation of the sample, but the range can be artificially inflated by one or two extreme values. Notice the extreme values in the sample of leaf measurements previously discussed. Moreover, ranges do not tell us anything about the measurements between the extremes.

Question 8

a. Could two samples have the same mean but different ranges? Explain.

Rounding Numbers

Do not change the value of the last significant digit if that digit is followed by a number that is less than 5. For example, if two significant figures are required, 6.449 rounds to 6.4, 66.449 rounds to 66, 66.641 rounds to 67, and 6.591 rounds to 6.6. Here is how an original measurement of 49.5149 rounds to various numbers of significant figures:

Five significant figures:	49.515
Four significant figures:	49.51
Three significant figures:	49.5
Two significant figures:	50
One significant figure:	50

Statisticians disagree on what to do when the number following the last significant figure is exactly 5, as in 89.5 (and, in this case, the precision is limited to two significant figures). Some round the measurement to the higher number, while others claim that doing so introduces bias into the data. You can decide which approach to take, but be consistent.

b. Could two samples have the same range but different means? Explain.

The **standard deviation** indicates how measurements vary about the mean. The standard deviation is easy to calculate. Begin by calculating the mean, measuring the deviation of each sample from the mean, squaring each deviation, and then summing the deviations. This summation results in the **sum of squared deviations.** For example, consider a group of shrimp that are 22, 19, 18, and 21 cm long. The mean length of these shrimp is 20 cm.

Sample Value	*Mean*	*Deviation*	*(Deviation)²*
22	20	2	4
19	20	−1	1
21	20	1	1
18	20	−2	4

Sum of Squared Deviations = 10

The summary equation for the sum of squared deviations is:

$$\textbf{Sum of squared deviations} = \sum_{i=1}^{N}(x_i - \bar{x})^2$$

where

N = **total number of samples**

$\bar{x}$ = **the sample mean**

x_i = **measurement of an individual sample**

This formula is simple. The summation sign ($\sum_{i=1}^{N}$) means to add up all the squared deviations from the first one ($i = 1$) to the last one ($i = N$). The sum of squared deviations (10) divided by the number of samples minus one (4 − 1 = 3) produces a value of 10/3 = 3.3 cm^2 (note that the units are centimeters squared). This is the **variance:**

$$\textbf{Variance} = \frac{\textbf{sum of squared deviations}}{N - 1}$$

The square root of the variance, 1.8 cm, equals the **standard deviation (SD):**

$$\textbf{SD} = \sqrt{\textbf{Variance}} = \sqrt{3.3} = 1.8$$

The standard deviation is usually reported with the mean in statements such as, “The mean length of the shrimp was 20 ± 1.8 cm.”

The standard deviation helps us understand the spread or variation of a sample. For many distributions of measurements, the mean ± 1 SD includes 68% of the measurements, whereas the mean ± 2 SD includes 95% of the measurements.

Procedure 2.7 **Gather and analyze data statistically**

1. Use a meterstick or tape measure to measure your height in centimeters. Record your height here: ___________________cm

2. Record your height and gender (male or female) on the board in the lab.

3. After all of your classmates have reported their heights, calculate the following:

Size of sample

All classmates _____________________

Male classmates ___________________

Female classmates _________________

Mean height

All classmates _____________________

Male classmates ___________________

Female classmates _________________

Median height

All classmates ____________________

Male classmates __________________

Female classmates ________________

Range

All classmates _________ to _________

Male classmates __________ to _________

Female classmates__________ to _________

Standard deviation

All classmates ± _________

Male classmates ± _________

Female classmates ± _________

If there is sufficient time, obtain a newspaper that advertises cars, groceries, or other common commodities. Choose one example (e.g., new cars) and determine its average price (e.g., determine the average price of a new car).

Question 9

a. What does your calculation tell you?

b. What are the limitations of your sample?

Your instructor may ask you to do other statistical tests, such as Student's *t*, chi-square, and analysis of variance (ANOVA). The type of test you'll do will depend on the amount and type of data you analyze, as well as the hypotheses you are trying to test.

INVESTIGATION

Variation in the Areas and Shapes of Leaves

Observation: Leaves, which are the primary photosynthetic organ of most plants, are adapted for absorbing light. This involves exposing large surface areas to the environment.

Question: How does the surface area and shape of leaves vary on different parts of plants?

a. Establish a working lab group and obtain Investigation Worksheet 2 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. If leaves are not available from outdoor plants (e.g., during winter), use the plants provided by your instructor that were grown indoors. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 2 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What are the advantages and disadvantages of using the metric system of measurements?

2. Why is it important for all scientists to use a standard system of measures rather than the system that may be most popular in their home country or region?

3. Do you lose or gain information when you use statistics to reduce a population to a few characteristic numbers? Explain your answer.

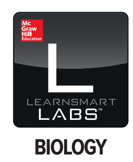
4. Suppose that you made repeated measurements of your height. If you used good technique, would you expect the range to be large or small? Explain your answer.

5. Suppose that a biologist states that the average height of undergraduate students at your university is 205 cm plus or minus a standard deviation of 17 cm. What does this mean?

6. What does a small standard deviation signify? What does a large standard deviation signify?

7. It is possible to make a perfectly precise measurement? Explain.

8. When in our everyday lives do we *not* want precise measurements?



Exercise 3

The Microscope

Basic Skills of Light Microscopy

Learning Objectives

By the end of this exercise you should be able to:

1. Identify and explain the functions of the primary parts of a compound microscope and dissecting (stereoscopic) microscope.
2. Carry and focus a microscope properly.
3. Use a compound microscope and dissecting microscope to examine biological specimens.
4. Prepare a wet mount, determine the magnification and size of the field of view, and determine the depth of field.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Many organisms and biological structures are too small to be seen with the unaided eye (fig. 3.1). Biologists often use a light microscope to observe such specimens. A **light microscope** is a coordinated system of lenses arranged to produce an enlarged, focusable image of a specimen. A light microscope **magnifies** a specimen, meaning that it increases its apparent size. Magnification with a light microscope is usually accompanied by improved **resolution,** which is the ability to distinguish two points as separate points. Thus, the better the resolution, the sharper or crisper the image appears. The resolving power of the unaided eye is approximately 0.1 mm (1 in = 25.4 mm), meaning that our eyes can distinguish two points that are 0.1 mm apart. A light microscope, used properly, can improve resolution as much as 1000-fold (i.e., to 0.1 µm).

The ability to discern detail also depends on **contrast,** which is the difference between the lightest and darkest parts of an image. Therefore, many specimens examined with a light microscope are stained with artificial dyes that increase contrast and make the specimen more visible.

The invention of the light microscope was profoundly important to biology, because it was used to formulate the cell theory and study biological structure at the cellular level. Light microscopy has revealed a vast new world to the human eye and mind (fig. 3.2). Today, the light microscope is the most fundamental tool of many biologists.

THE COMPOUND LIGHT MICROSCOPE

Study and learn the parts of the typical compound light microscope shown in figure 3.3. A light microscope has two, sometimes three, systems: an illuminating system, an imaging system, and possibly a viewing and recording system.

Illuminating System

The illuminating system, which concentrates light on the specimen, usually consists of a light source, condenser lens, and iris diaphragm. The **light source** is a lightbulb located at the base of the microscope. The light source illuminates the specimen by passing light through a thin, almost transparent part of the specimen. The **condenser lens,** located immediately below the specimen, focuses light from the

Caring for Your Microscope

Microscopes are powerful tools for understanding biology. However, they're also expensive and fragile and require special care. When you use your microscope, always do the following to ensure optimal performance and care:

- Always carry your microscope upright with both hands—one hand under the base and the other around the microscope's arm (fig. 3.3).
- Always begin by cleaning the ocular and objective lenses with lens paper.
- Always start your examinations with the low-power objective in place.
- If you shift to the high-power objective, rotate the objective into place carefully. Never force the objective lens into place. If the objective lens contacts the slide, stop and restart your examination with the low-power objective lens.
- After shifting to the high-power objective, always use only the fine adjustment to focus the image.
- When you've completed your work with the microscope, clean the lenses with lens paper, wrap the electrical cord securely around the microscope's arm, and return your microscope to its storage area.

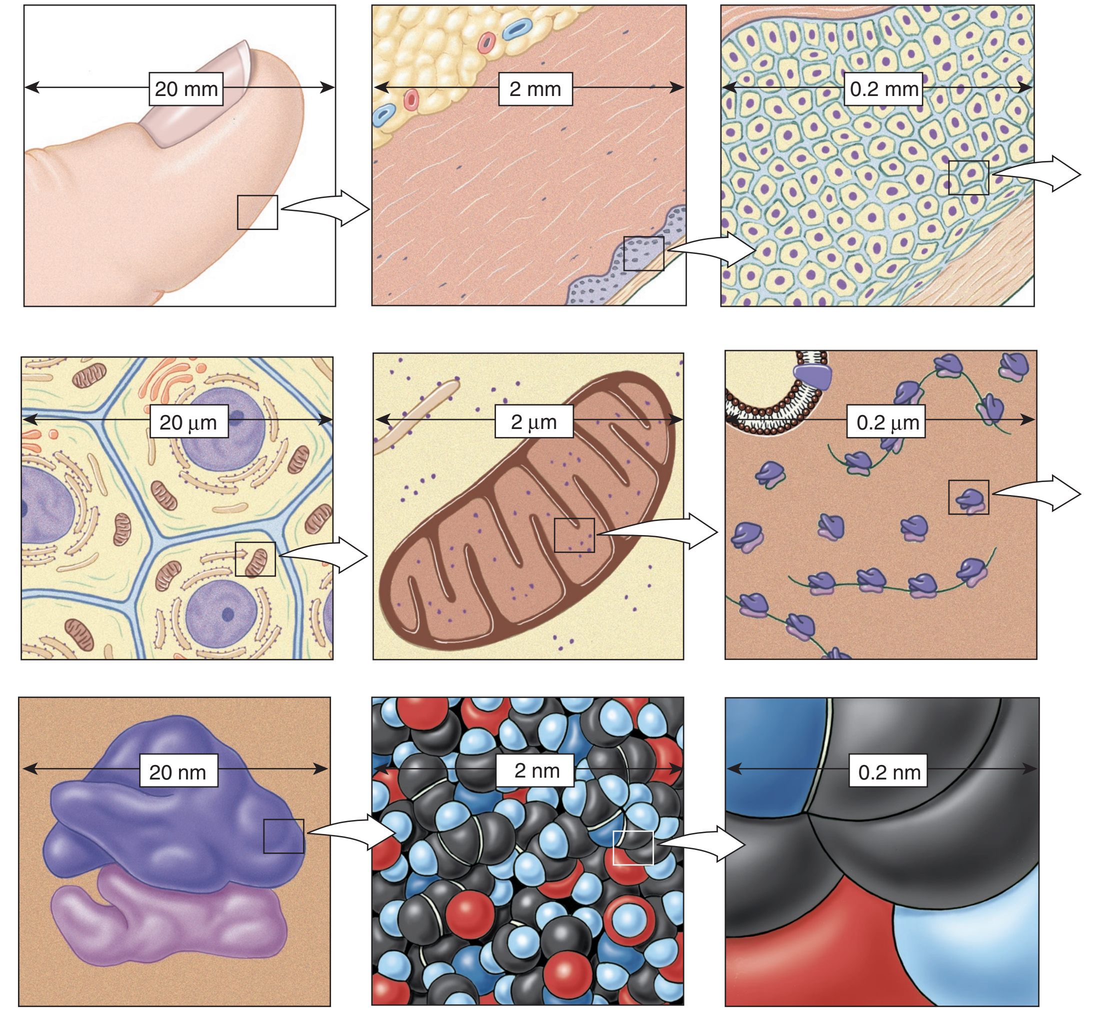


Figure 3.1 The size of cells and their contents. This diagram shows the size of human skin cells, organelles, and molecules. In general, the diameter of a human skin cell is about 20 micrometers (µm), of a mitochondrion is 2 µm, of a ribosome is 20 nanometers (nm), of a protein molecule is 2 nm, and of an atom is 0.2 nm.

light source onto the specimen. Just below the condenser is the **condenser iris diaphragm,** a knurled ring or lever that can be opened and closed to regulate the amount of light reaching the specimen. When the condenser iris diaphragm is open, the image will be bright; when closed, the image will be dim.

Imaging System

The imaging system improves resolution and magnifies the image. It consists of the objective and ocular (eyepiece) lenses and a body tube. The objectives are three or four lenses mounted on a revolving nosepiece. Each objective is a series of several lenses that magnify the image, improve resolution, and correct aberrations in the image. The most common configuration for student microscopes includes four objectives: low magnification (4×), medium magnification (10×), high magnification (40×), and oil immersion (100×). Using the oil immersion objective requires special instructions, as explained in Exercise 24 to study bacteria. To avoid damaging your microscope, do not use the oil immersion objective during this exercise.

The magnifying power of each objective is etched on the side of the lens (e.g., 4×). The **ocular** is the lens that you look through. Microscopes with one ocular are **monocular** microscopes, and those with two are **binocular** microscopes. Oculars usually magnify the image 10 times. The **body tube** is a metal casing through which light passes to the oculars. In microscopes with bent body-tubes and inclined oculars, the body tube contains mirrors and a prism that redirects light to the oculars. The **stage** secures the glass slide on which the specimen is mounted.

Viewing and Recording System

The viewing and recording system, if present, converts radiation to a viewable and/or permanent image. The viewing and recording system usually consists of a camera or video screen. Most student microscopes do not have viewing and recording systems.



© Heritage Image Partnership Ltd/Alamy

Figure 3.2 "Egad, I thought it was tea, but I see I've been drinking a blooming micro-zoo!" says this horrified, proper nineteenth-century London woman when she used a microscope to examine her tea. People were shocked to learn that there is an active, living world too small for us to see.

Oculars
Body tube
Arm
Slide holder to adjust position
Coarse focus adjustment
Fine focus adjustment
Nosepiece
Objectives
Stage
Stage clip
Condenser
Condenser iris diaphragm
Condenser adjustment
Light source
Base

© BiologyImaging.com

Figure 3.3 Major parts of a compound light microscope.

A Summary of How to Use a Compound Light Microscope

1. Place the specimen on the microscope's stage.
2. Rotate the low-power objective into place. Center the specimen below the objective.
3. Look through the oculars while using the coarse adjustment to focus on the specimen. Center the area of the specimen that you want to examine.
4. Slowly rotate the high-power objective into place. Look through the oculars while you use the fine adjustment to focus on the specimen.
5. If you "lose" your specimen when you switch from low power to high power, retrace the previous steps, paying special attention to placing the specimen in the center of the field of view.

USING A COMPOUND MICROSCOPE

Although the maximum magnification of light microscopes has not increased significantly during the last century, the construction and design of light microscopes have improved the resolution of newer models. For example, built-in light sources have replaced adjustable mirrors in the illuminating system, and lenses are made of better glass than they were in the past.

Your lab instructor will review with you the parts of the microscopes (and their functions) you will use in the lab (fig. 3.3). After familiarizing yourself with the parts of a microscope, you're now ready for some hands-on experience with the instrument. The videos at the website associated with this manual (connect.mheducation.com) will be especially useful for helping you understand how to properly use your microscope.

Procedure 3.1 Use a compound microscope

1. Remove the microscope from its cabinet and carry it upright with one hand grasping the arm and your other hand supporting the microscope below its base. Place your microscope on the table in front of you.



Do not use paper towels or Kimwipes to clean the lenses of your microscope; they can scratch the lenses. Clean the lenses only with lens paper.

2. Plug in the microscope and turn on the light source.

3. If it isn't already in position, rotate the nosepiece until the scanning (4×) objective is in line with the light source. (The 4× objective is called the "scanning objective" because it enables users to scan large areas of a specimen.) You'll feel the objective click into place when it is positioned properly. *Always begin examining slides with the scanning objective.*

4. Locate the coarse adjustment knob on the side of the microscope. Depending on the type of microscope that you're using, the coarse adjustment knob moves either the nosepiece (with its objectives) or the stage to focus the lenses on the specimen. Only a partial turn of the coarse adjustment knob moves the stage or nosepiece a relatively large distance. *The coarse adjustment should only be used when you're viewing a specimen with the 4× or 10× objective lens.*

5. If your microscope is binocular, adjust the distance between the oculars to match the distance between your pupils. If your microscope is monocular, keep both eyes open when using the microscope. After a little practice you will ignore the image received by the eye not looking through the ocular.

6. Focus a specimen by using the following steps:

- ***a.*** Place a microscope slide of newsprint of the letter *e* on the horizontal stage so that the *e* is directly below the scanning objective lens and is right side up. It should be centered over the hole in the stage.
- ***b.*** Rotate the coarse adjustment knob to move the objective within 1 cm of the stage (1 cm = 0.4 in).
- ***c.*** Look through the oculars with both eyes open.
- ***d.*** Rotate the coarse adjustment knob (i.e., raising the objective lens or lowering the stage) until the *e* comes into focus. If you don't see an image, the *e* is probably off center. Be sure that the *e* is directly below the objective lens and that you can see a spot of light surrounding the *e*.
- ***e.*** Focus up and down to achieve the crispest image.
- ***f.*** Adjust the condenser iris diaphragm so that the brightness of the transmitted light provides the best view.
- ***g.*** Observe the letter, then rotate the nosepiece to align the 10× objective to finish your observation. Do not use the oil immersion objective.

Question 1

a. As you view the letter *e,* how is it oriented? Upside down or right side up?

b. How does the image move when the slide is moved to the right or left? Toward you or away from you?

c. What happens to the brightness of the view when you go from 4× to 10×?

Magnification

Procedure 3.2 **Determine magnification**

1. Estimate the magnification of the *e* by looking at the magnified image on lowest magnification (4×), and then at the *e* without using the microscope.
2. Examine each objective and record the magnifications of the objectives and oculars of your microscope in table 3.1.
3. Calculate and record in table 3.1 the total magnification for each objective following this formula:

$$\mathbf{Mag_{Tot} = Mag_{Obj} \times Mag_{Ocu}}$$

where

$\mathbf{Mag_{Tot}}$ **= total magnification of the image**

$\mathbf{Mag_{Obj}}$ **= magnification of the objective lens**

$\mathbf{Mag_{Ocu}}$ **= magnification of the ocular lens**

For example, if you're viewing the specimen with a 4× objective lens and a 10× ocular, the total magnification of the image is 4 × 10 = 40×. That is, the specimen appears 40 times larger than it is.

4. Slowly rotate the high-power (i.e., 40×) objective into place. *Be sure that the objective does not touch the slide!* If the objective does not rotate into place without touching the slide, do not force it; ask your lab instructor to help you. After the 40× objective is in place, you should notice that the image remains near focus. Most light microscopes are **parfocal,** meaning that the image will remain nearly focused after the 40× objective lens is moved into place. Most light microscopes are also **parcentered,** meaning that the image will remain centered in the field of view after the 40× objective lens is in place.
5. You may need to readjust the iris diaphragm because the high-magnification objective allows less light to pass through to the ocular.
6. To fine-focus the image, locate the **fine adjustment knob** on the side of the microscope. Turning this knob changes the specimen-to-objective distance slightly and therefore makes it easy to fine-focus the image. **Use only the fine adjustment when using the 40× (or higher) objective.**



Never use the coarse adjustment knob to focus an image on high power.

7. Compare the size of the image under high magnification with the image under low magnification.

Question 2

a. How many times is the image of the *e* magnified when viewed through the high-power objective?

b. If you didn't already know what you were looking at, could you determine at this magnification that you were looking at a letter *e*? How?

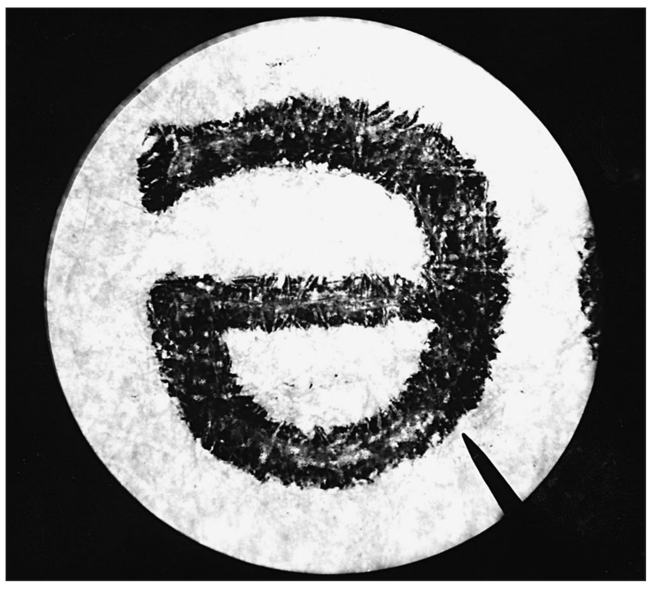
Determine the Size of the Field of View

The **field of view** is the area that you can see through the ocular and objective (fig. 3.4). Knowing the size of the field of view is important because you can use it to estimate the size of an object you are examining. The field of view can be measured with ruled **micrometers** (fig. 3.5). An **ocular micrometer** is a small glass disk with thin lines numbered and etched in a row. It was put into an ocular on your microscope so that the lines superimpose on the image and allow you to measure the specimen. Before you can use the micrometer you must determine for each magnification the apparent distance between the lines on the ocular micrometer. This means that you must calibrate the ocular micrometer by comparing its lines to those lines on a

TABLE 3.1

TOTAL MAGNIFICATIONS AND AREAS OF FIELD OF VIEW (FOV) FOR THREE OBJECTIVES

Objective Power	Objective Magnification	×	Ocular Magnification	=	Total Magnification	FOV Diameter (mm)	FOV Area (mm²)	Measurement (mm) for 1 Ocular Space
4×	________	×	________	=	________	________	________	________
10×	________	×	________	=	________	________	________	________
40×	________	×	________	=	________	________	________	________



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Figure 3.4 The circular, illuminated field of view of a compound light microscope. Shown here is the letter *e* from newsprint that is magnified 40 times (i.e., 40×).

standard ruler called a **stage micrometer.** A stage micrometer is a glass slide having precisely spaced lines etched at known intervals.

Procedure 3.3 **Use a stage micrometer to calibrate the ocular micrometer, and determine the size of the field of view**

1. Rotate the ocular until the lines of the ocular micrometer parallel those of the stage micrometer (fig. 3.5).
2. Align lines at the left edges (0 lines) of the two micrometers by moving the stage micrometer (fig. 3.5).
3. Count how many spaces on the stage micrometer fit precisely in a given number of spaces on the ocular micrometer. Record the values here.

y ocular spaces = x stage spaces

y =

x =

View of ocular micrometer

View of stage micrometer

View of both micrometers aligned at their 0 lines

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Figure 3.5 Stage and ocular micrometers. Micrometers are used to calibrate microscopes and measure the size of specimens.

The smallest space on a stage micrometer = 0.01 mm, so

$$y \text{ ocular spaces (mm)} = x \text{ stage spaces} \times 0.01$$

$$1 \text{ ocular space (mm)} = (x/y) \times 0.01$$

4. Calculate the distance in millimeters between lines of the ocular micrometer. For example, if the length of 10 spaces on the ocular micrometer equals the length of seven spaces on the stage micrometer, then

$$y = 10$$
$$x = 7$$
$$10 \text{ ocular spaces (mm)} = 7 \text{ stage spaces} \times 0.01 \text{ mm}$$
$$1 \text{ ocular space (mm)} = (7 \times 0.01 \text{ mm})/10$$
$$1 \text{ ocular space (mm)} = 0.007 \text{ mm}$$
$$1 \text{ ocular space} = 7\ \mu\text{m}$$

   Therefore, if a specimen spans eight spaces on your ocular micrometer with that objective in place, that specimen is 56 µm long.
5. Calibrate the ocular micrometer for each objective on your microscope. Record in table 3.1 the diameter of the field of view (FOV) for each objective. Also record for each objective lens in table 3.1 the measurement (mm) for 1 ocular space. You can use this information in future labs as you measure the sizes of organisms and their parts.
6. Calculate the radius, which is half the diameter.
7. Use this information to determine the area of the circular field of view with the following formula:

$$\text{Area of circle} = \pi \times \text{radius}^2$$
$$(\pi = 3.14)$$

8. Record your calculated FOV areas in table 3.1.

Alternate Procedure 3.3 Use a transparent ruler to determine the size of the field of view

1. Obtain a clear plastic ruler with a metric scale.
2. Place the ruler on the stage and under the stage clips of your microscope. If your microscope has a mechanical stage, ask your instructor how to place the ruler to avoid damage. Carefully rotate the nosepiece to the objective of lowest magnification.
3. Slowly focus with the coarse adjustment, and then with the fine adjustment, until the metric markings on the ruler are clear.
4. Align the ruler to measure the diameter of the circular field of view. The space between each line on the ruler should represent a 1-mm interval.
5. Record in table 3.1 the diameter of this low-magnification field of view. Also calculate the radius, which is half the diameter.
6. The ruler cannot be used to measure the diameters of the field of view at medium and high magnifications because the markings are too far apart. Therefore, these diameters must be calculated using the following formula:

$$FOV_{low} \times Mag_{low} = FOV_{high} \times Mag_{high}$$

   where

   FOV_{low} = **diameter of the field of view of the low-power objective**

   Mag_{low} = **magnification of the low-power objective (Be consistent and use the magnification of the objective, not total magnification.)**

   FOV_{high} = **diameter of the field of view of the high-power objective**

   Mag_{high} = **magnification of the high-power objective**

   For example, if 3.0 mm is the diameter of the field of view for a 4× low-power objective, then what is the diameter of the field of view of the 40× high-power objective?

$$3.0 \text{ mm} \times 4 = FOV_{high} \times 40$$
$$0.30 \text{ mm} = FOV_{high}$$

7. Calculate and record in table 3.1 the diameters of the field of view for the 10× and 40× magnifications.
8. Calculate and record in table 3.1 the circular area of the field of view for the three magnifications by using the following formula.

$$\text{Area of circle} = \pi \times \text{radius}^2$$
$$(\pi = 3.14)$$

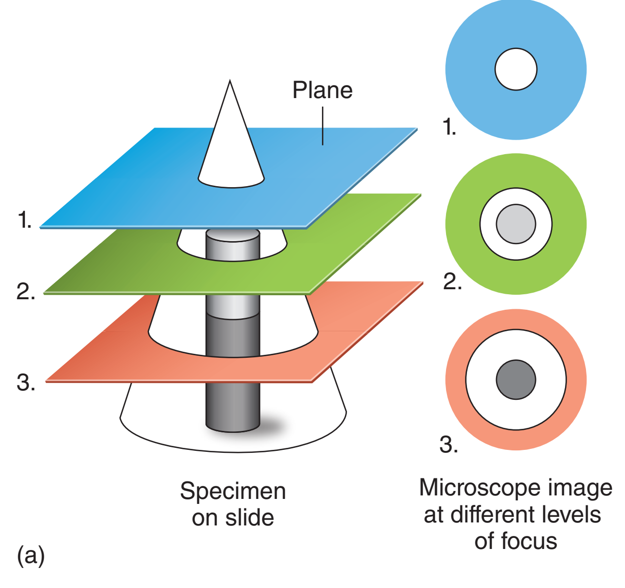
Question 3

a. Which provides the largest field of view, the 10× or 40× objective?

b. How much more area can you see with the 4× objective than with the 40× objective?

c. Why is it more difficult to locate an object starting with the high-power objective than with the low-power objective?

d. Which objective should you use to initially locate the specimen? Why?





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Figure 3.6 How focusing at different planes of a specimen would produce three different images. (*a*) Focusing up and down when you view a specimen can help you to understand its three-dimensional structure. (*b*) A thin depth of field is apparent in this 100× image of cells of *Closterium*, a green alga. The upper and lower layers of cells are out of focus, while the midlayer of cells is within the thin depth of field and is clearly focused.

Determine the Depth of Field

Depth of field is the thickness of the object in sharp focus (fig. 3.6). Depth of field varies with different objectives and magnifications.

Procedure 3.4 **Determine the depth of the field of view**

1. Using the low-power objective, examine a prepared slide of three colored threads mounted on top of each other.

2. Focus up and down and try to determine the order of the threads from top to bottom. The order of the threads will not be the same on all slides.

3. Re-examine the threads using the high-power objective lens.

Question 4

a. Are all three colored threads in focus at low power?

b. Can all three threads be in focus at the same time using the high-power objective?

c. Which objective, high or low power, provides the greatest depth of field?

Preparing a Wet Mount of a Biological Specimen

Procedure 3.5 **Prepare a wet mount of a biological specimen**

1. Place a drop of water containing algal cells from a culture labeled “Algae” on a clean microscope slide.

2. Place the edge of a clean coverslip at an edge of the drop at a 45° angle; then slowly lower the coverslip onto the drop so that no air bubbles are trapped (fig. 3.7). (Your instructor will demonstrate this technique.) This fresh preparation is called a **wet mount** and can be viewed with your microscope.

3. Experiment with various intensities of illumination. To do this, rotate the 4× objective into place and adjust the condenser iris diaphragm to produce the least illumination. Observe the image; note its clarity, contrast, and color. Repeat these observations with at least four different levels of illumination. The fourth level should have the diaphragm completely open.

4. Repeat step 3 for the 10× and 40× objectives.

Question 5

a. Is the image always best with highest illumination?

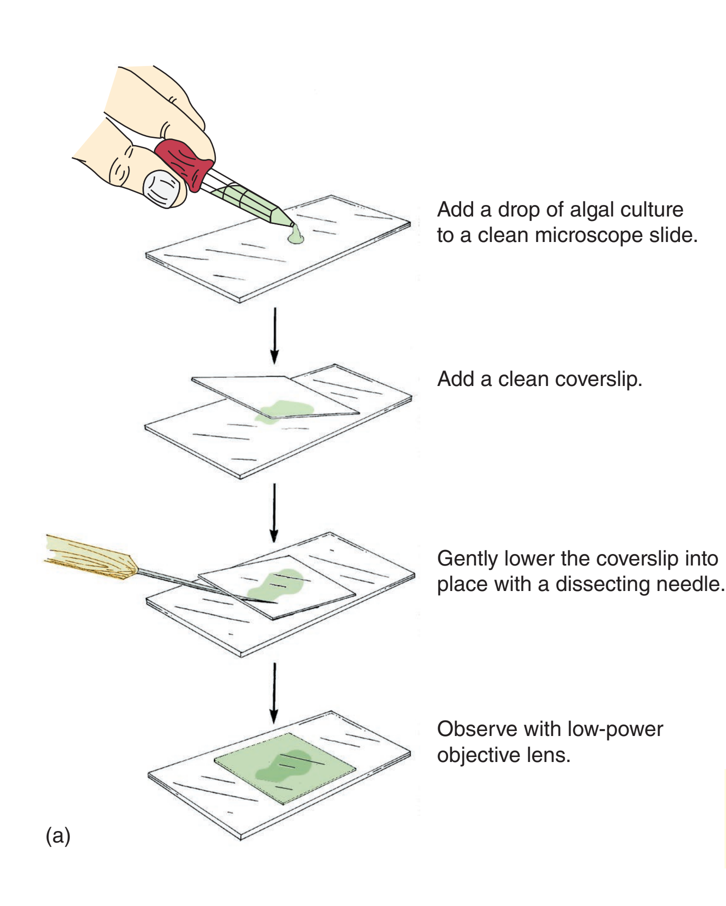




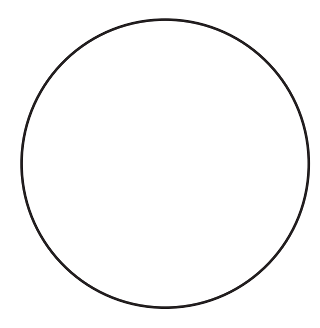
Figure 3.7 (*a*) Preparing a wet mount of a biological specimen. (*b*) A wet mount of pond water will often include the common cyanobacterium *Oscillatoria* (200×). See also figures 3.6 and 25.1–25.4.



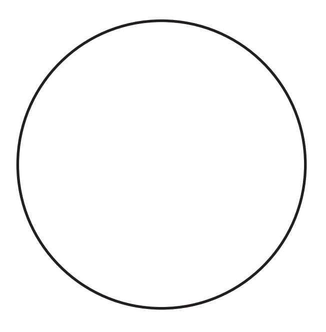
b. Is the same level of illumination best for all magnifications?

c. Which magnifications require the most illumination for best clarity and contrast?

5. Examine your preparation of algae, and sketch in the following field of view the organisms that you see. Don't mistake air bubbles for organisms! Air bubbles appear as uniformly round structures with dark, thick borders.



6. Prepare a wet mount of some newly hatched brine shrimp (*Artemia,* which are popularly referred to as "sea monkeys") and their eggs. Sketch in the following field of view what you see. Use your calculations for the diameter of the field of view to estimate the length of the shrimp.



Approximate length of the shrimp: ________

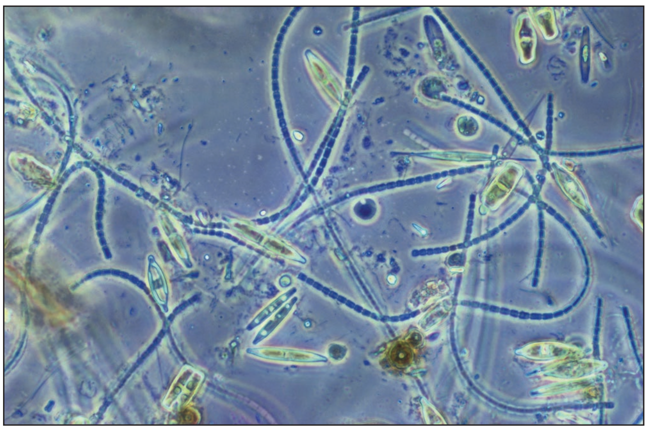
Question 6

a. Why is it important to put a coverslip over the drop of water when you prepare a wet mount?

b. Approximately how long and wide is a brine shrimp?

Practice

For practice using your microscope, prepare some wet mounts of pond water or a hay infusion to view the diversity of protozoa and algae (fig. 3.8). If the protozoa are moving too fast for you to examine carefully, add a drop of methylcellulose (often sold commercially as Proto-Slo) to your



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Figure 3.8 The diversity of organisms in pond water (200×).

sample. (The methylcellulose will slow the movement of the protozoa.) Also examine the prepared slides available in the lab. You'll examine these slides in more detail in the coming weeks, so don't worry about their contents. Rather, use this exercise to familiarize yourself with the microscope. Also prepare wet mounts of the cultures available in the lab and sketch the organisms that you see. When you've finished, turn off the light source, cover your microscope, and store the microscope in its cabinet.

THE DISSECTING (STEREOSCOPIC) MICROSCOPE

A **dissecting (stereoscopic) microscope** offers some advantages over a compound microscope. Although a compound microscope can produce high magnifications and excellent resolution, it has a small **working distance,** which is the distance between the objective lens and specimen. Therefore, it is difficult to manipulate a specimen while observing it with a compound microscope. Specimens that can be observed with a compound microscope are limited to those thin enough for light to pass through them. In contrast, a dissecting microscope is used to view objects that are opaque or too large to see with a compound microscope.

A dissecting microscope provides a much larger working distance than does a compound microscope. This distance is usually several centimeters (compared to a centimeter or less for a compound microscope), making it possible to dissect and manipulate most specimens. Also, most specimens for dissection are too thick to observe with transmitted light from a light source below the specimen. Therefore, many dissecting microscopes use a light source above the specimen; the image you see is formed from reflected light.

Dissecting microscopes are always binocular (fig. 3.9). Each ocular views the specimen at different angles through one or more objective lenses. This arrangement provides a three-dimensional image with a large depth of field. This is in contrast to the image in a compound microscope, which is basically two-dimensional. However, the advantages of a stereoscopic microscope are often offset by lower resolution and magnification than a compound microscope. Most dissecting microscopes have magnifications of 4× to 50×.

Procedure 3.6 Use a dissecting microscope

1. Carry the dissecting microscope to your desk.
2. Use figure 3.9 to familiarize yourself with the parts of your microscope.
3. Use your dissecting microscope to examine the organisms available in the lab. Sketch some of these organisms.
4. Use a ruler to measure the diameter of the field of view with your dissecting microscope at several levels of magnification.

Question 7

a. What is the area of the field of view when you use the lowest magnification of your dissecting microscope?

b. What is the area when you use the highest magnification?

c. Place a microscope slide of the letter *e* on the stage. As you view the letter *e,* how is it oriented?

d. How does the image through a dissecting microscope move when the specimen is moved to the right or left? Toward you or away from you?

e. How does the direction of illumination differ in dissecting as opposed to compound microscopes?

A COMPARISON OF COMPOUND AND DISSECTING MICROSCOPES

Complete table 3.2 comparing magnification, resolution, size of the field of view, and depth of field of a dissecting microscope and a compound microscope. Use the terms *high, low,* or *same* to describe your comparisons.

Question 8

What other differences are there between compound and dissecting microscopes?

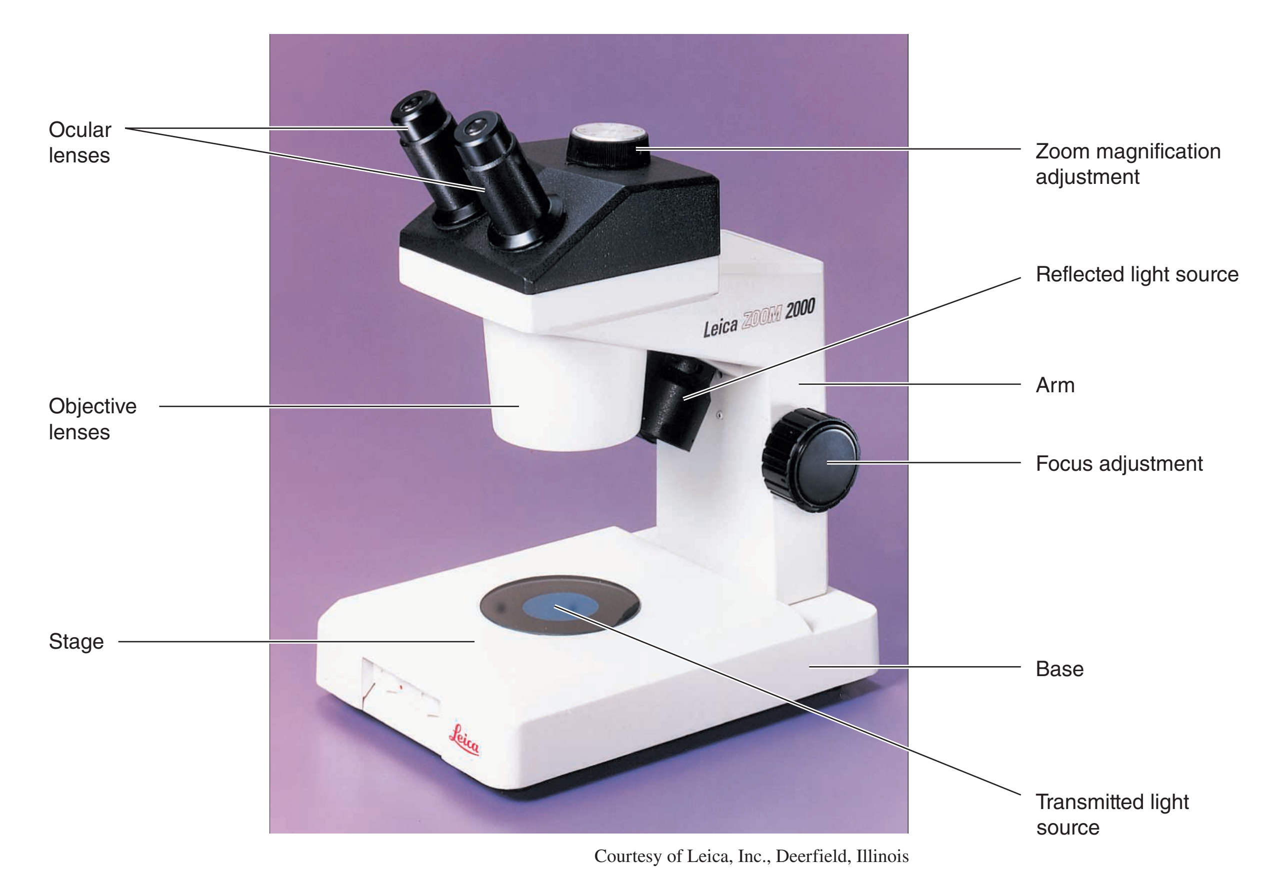


Figure 3.9 Major parts of a dissecting (stereoscopic) microscope.

Table 3.2

A Comparison of Dissecting and Compound Microscopes

Characteristic	Dissecting Microscope	Compound Microscope
Magnification	____________	____________
Resolution	____________	____________
Size of field of view	____________	____________
Depth of field	____________	____________

INVESTIGATION

The Shapes, Surface Areas, and Volumes of Red Blood Cells

Observation: Red blood cells, which are the most common type of blood cell, are used by vertebrates to deliver oxygen to body tissues. Red blood cells are filled with hemoglobin, which gives them their characteristic color.

Question: What are the shapes, surface areas, and volumes of red blood cells?

a. Establish a working lab group and obtain Investigation Worksheet 3 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 3 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What are the advantages of knowing the diameter of the field of view at a given magnification?

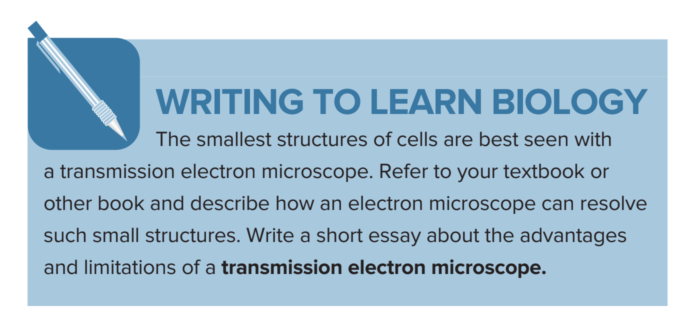
2. Why must specimens viewed with a compound microscope be thin? Why are they sometimes stained with dyes?

3. Why is depth of field important in studying biological structures? How can it affect your ability to find and examine a specimen?

4. What is the importance of adjusting the light intensity when viewing specimens with a compound microscope?

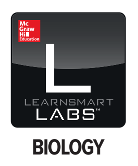
5. What is the function of each of the following parts of a compound and dissecting microscope?
Oculars
Objectives
Condenser
Iris diaphragm
Stage
Coarse adjustment
Fine adjustment

6. Examine the micrograph of the letter *e* shown in figure 3.4. This letter is magnified 40×. What is the actual height of the letter?



WRITING TO LEARN BIOLOGY

The smallest structures of cells are best seen with a transmission electron microscope. Refer to your textbook or other book and describe how an electron microscope can resolve such small structures. Write a short essay about the advantages and limitations of a **transmission electron microscope.**



Exercise 4

The Cell

Structure and Function

Learning Objectives

By the end of this exercise you should be able to:

1. Understand the differences between prokaryotes and eukaryotes and identify structures characteristic of each.
2. Prepare a wet mount to view cells with a compound microscope.
3. Explain the function of organelles within eukaryotic cells visible with a light microscope.
4. Examine a cell's structure and determine whether it is from a plant or animal.
5. Observe representatives of the protists, a large group of eukaryotic organisms that are heterotrophic or autotrophic.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Cells are the basic unit of living organisms because they perform all of the processes we collectively call "life." All organisms are made of cells. Although most individual cells are visible only with the aid of a microscope, some may be a meter long (e.g., nerve cells) or as large as a small orange (e.g., the yolk of an ostrich egg). Despite these differences, all cells are designed similarly and share fundamental features.

Cytology is the study of cellular structure and function. The major tools of cytologists are light microscopy, electron microscopy, and cell chemistry. By studying the anatomy of a cell, we can find clues to how the cell works.

In today's lab you will study some of the features and variations among living cells. Prior to this exercise, review in your textbook the general features of cellular structure and function.

PROKARYOTIC CELLS

Bacteria and cyanobacteria are **prokaryotes** (fig. 4.1), and their diversity is considerable (>5000 species). Prokaryotes

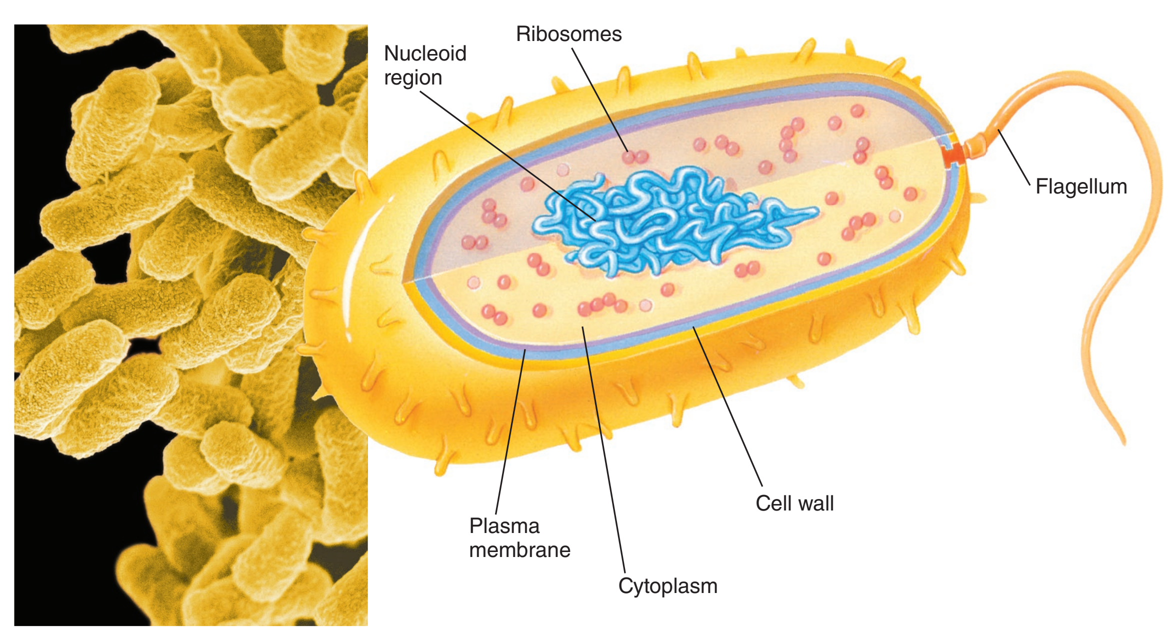


Figure 4.1 The structure of a bacterial cell. Bacteria lack a nuclear membrane. All prokaryotic (bacterial) cells have a nucleoid region, ribosomes, plasma membrane, cytoplasm, and cell wall, but not all have flagella (1500×) Many bacterial cells are surrounded by a gelatinous capsule and have pili as well as flagella.

do not contain a membrane-bound nucleus or any other membrane-bound **organelles.** Organelles are organized structures of macromolecules having specialized functions and are suspended in the **cytoplasm.** The cytoplasm of prokaryotes is enclosed in a **plasma membrane** (cellular membrane) and is surrounded by a supporting **cell wall** covered by a gelatinous **capsule. Flagella** and hairlike outgrowths called **pili** are common in prokaryotes; flagella are used for movement, and pili are used to attach some types of bacteria to surfaces or to exchange genetic material with other bacteria. Within the cytoplasm are **ribosomes** (small particles involved in protein synthesis) and **nucleoid regions** (concentrations of DNA). Prokaryotes do not reproduce sexually, but they have mechanisms for genetic recombination (see Exercise 16).

Cyanobacteria

The largest prokaryotes are **cyanobacteria,** formerly called blue-green algae. They contain chlorophyll *a* and accessory pigments for photosynthesis, but these pigments are not contained in membrane-bound chloroplasts. Instead, the pigments are held in photosynthetic membranes called **thylakoids** (fig. 4.2). Cyanobacteria are often surrounded by a **mucilaginous sheath.** Their ability to photosynthesize made them the primary contributors to the early oxygenation of the ancient earth's atmosphere.

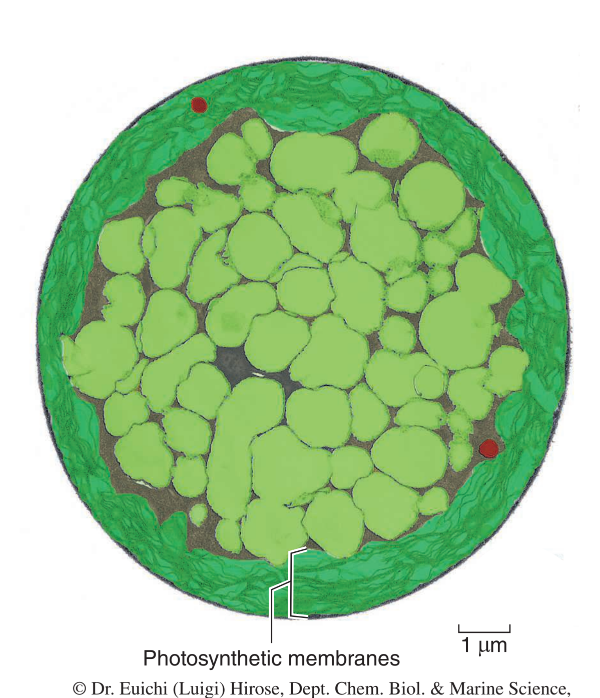


Figure 4.2 Electron micrograph of a photosynthetic bacterial cell, *Prochloron,* showing extensively folded photosynthetic membranes. The DNA is in the clear area in the central region of the cell; it is not membrane-bound (5200×).



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 4.1 Examine cyanobacteria

1. Examine a prepared slide of *Oscillatoria,* a filament of cells, and one of *Gloeocapsa,* a loosely arranged colony (fig 4.3). Review Exercise 3 and the associated videos for the proper way to use the microscope.
2. Focus with the low-power objective.
3. Rotate the high-power objective into place to see filaments and masses of cells.

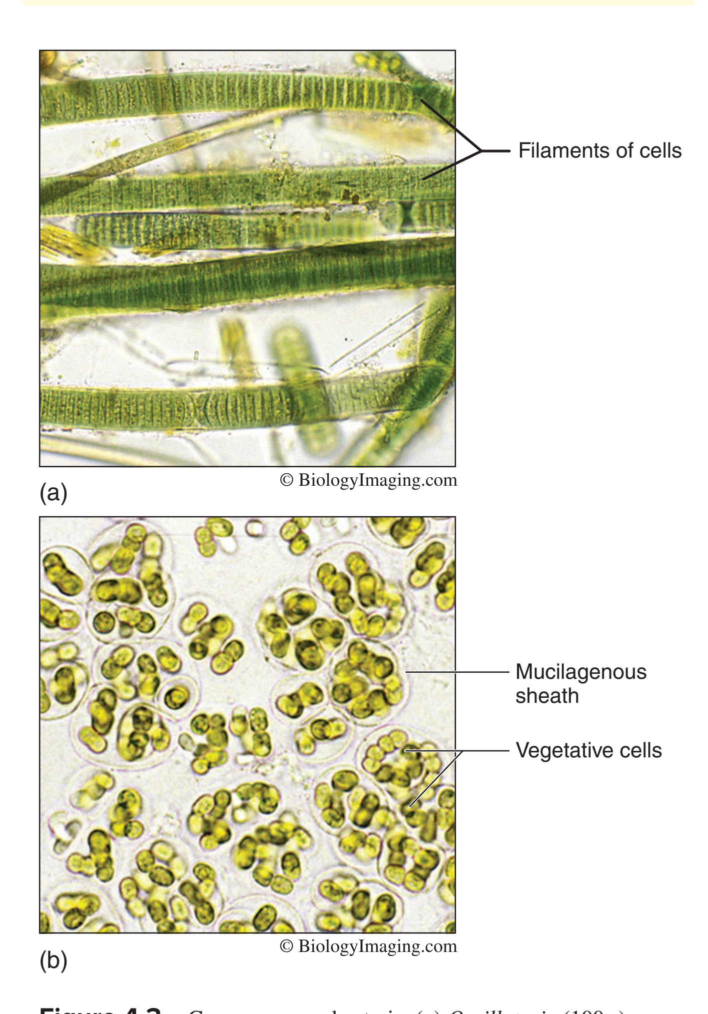


Figure 4.3 Common cyanobacteria. (*a*) *Oscillatoria* (100×). (*b*) *Gloeocapsa* (400×).

4. Prepare a wet mount of *Oscillatoria* and one of *Gloeocapsa.* Review procedure 3.5 in Exercise 3 for preparing a wet mount.
5. Observe the cellular structures and draw the cellular shapes and relative sizes of *Oscillatoria* and *Gloeocapsa* in the following space. Use an ocular micrometer to measure their dimensions.

Oscillatoria *Gloeocapsa*

Question 1

a. Where are the pigments located in these cyanobacteria?

b. Are nuclei visible in cyanobacterial cells?

c. Which of these two genera has the most prominent mucilaginous sheath?

d. How many cells are held within one sheath of *Gloeocapsa?*

Bacteria

Most bacteria are much smaller than cyanobacteria and do not contain chlorophyll. Yogurt is a nutrient-rich culture of bacteria. The bacterial cells composing most of the yogurt are *Lactobacillus,* a bacterium adapted to live on milk sugar (lactose). *Lactobacillus* converts milk to yogurt. Yogurt is acidic and keeps longer than milk. Historically, *Lactobacillus* has been used in many parts of the world by peoples deficient in lactase, an enzyme that breaks down lactose. Many Middle Eastern and African cultures use the more digestible yogurt in their diets instead of milk.

Procedure 4.2 Examine bacteria

1. Place a tiny dab of yogurt on a microscope slide.
2. Mix this small amount of yogurt in a drop of water, add a coverslip, and examine the yogurt with a compound microscope. Review Exercise 3.
3. Focus with the low-power objective.
4. Rotate the high-power objective (40×) into place to see masses of rod-shaped cells.
5. Observe the simple, external structure of the bacteria and draw their cellular shapes in the following space:

Question 2

How does the size of *Lactobacillus* compare with that of *Oscillatoria* and *Gloeocapsa?*

EUKARYOTIC CELLS

Eukaryotic cells are structurally more complex than prokaryotic cells. Although some features of prokaryotic cells are in eukaryotic cells (e.g., ribosomes, cell membrane), eukaryotic cells also contain several organelles not found in prokaryotic cells (table 4.1).

Eukaryotic cells contain membrane-bound **nuclei** and other organelles (figs. 4.4, 4.5). Nuclei contain genetic material of a cell and control metabolism. **Cytoplasm** forms the matrix of the cell and is contained by the plasma membrane. Within the cytoplasm are a variety of organelles. **Chloroplasts** are elliptical green organelles in plant cells. Chloroplasts are the site of photosynthesis in plant cells and are green because they contain chlorophyll, a photosynthetic pigment capable of capturing light energy. **Mitochondria** are organelles found in plant and animal cells. These organelles are where aerobic respiration occurs. When viewed with a conventional light microscope, mitochondria are small, dark, and often difficult to see. All of the material and organelles contained by the plasma membrane are collectively called the **protoplast.**

PLANT CELLS

Procedure 4.3 Examine living *Elodea* cells and chloroplasts

1. Remove a young leaf from the tip of a sprig of *Elodea. Elodea* is a common pond-weed used frequently in studies of photosynthesis, cellular structure, and cytoplasmic streaming.
2. Place this leaf, with the top surface facing up, in a drop of water on a microscope slide. The cells on the upper surface are larger and more easily examined. Add a coverslip, but do not let the leaf dry. Add another drop of water if necessary.

Table 4.1

Some of the Major Differences between Prokaryotic and Eukaryotic Cells and between Plant and Animal Cells

	Prokaryote	Eukaryote: Animal	Eukaryote: Plant
Exterior Structures			
Cell wall	Present (protein-polysaccharide)	Absent	Present (cellulose)
Cell membrane	Present	Present	Present
Flagella	May be present (single strand)	May be present	Absent except in sperm of a few species
Interior Structures			
ER	Absent	Usually present	Usually present
Ribosomes	Present	Present	Present
Microtubules	Absent	Present	Present
Centrioles	Absent	Present	Absent
Golgi complex	Absent	Present	Present
Other Organelles			
Nucleus	Absent	Present	Present
Mitochondria	Absent	Present	Present
Chloroplasts	Absent	Absent	Present
Chromosomes	A single circle of naked DNA	Multiple; DNA-protein complex	Multiple; DNA-protein complex
Vacuoles	Absent	Absent or small	Usually a large single vacuole

3. Examine the leaf with your microscope. Review Exercise 3 and the associated videos. First use low, then high, magnification to bring the upper layer of cells into focus (fig. 4.6). Each of the small, regularly shaped units you see are cells surrounded by cell walls made primarily of **cellulose** (fig. 4.7). Cellulose is a complex carbohydrate made of glucose molecules attached end-to-end. The plasma membrane lies just inside the cell wall. Sketch what you see.

Question 3

a. What three-dimensional shape are *Elodea* cells?

b. Examine various layers of cells by focusing up and down through the layers. About how many cells thick is the leaf that you are observing?

c. What are the functions of the cell wall?

d. Use an ocular micrometer or refer to the dimensions of the field of view calculated in Exercise 3 to measure the dimensions of an *Elodea* cell. What are the cell's approximate dimensions?

4. Chloroplasts appear as moderately sized green spheres within the cells (figs. 4.6, 4.8). Locate and sketch cells having many chloroplasts; estimate the number of chloroplasts in a healthy cell. Remember that a cell is three-dimensional, and some chloroplasts may obscure others.

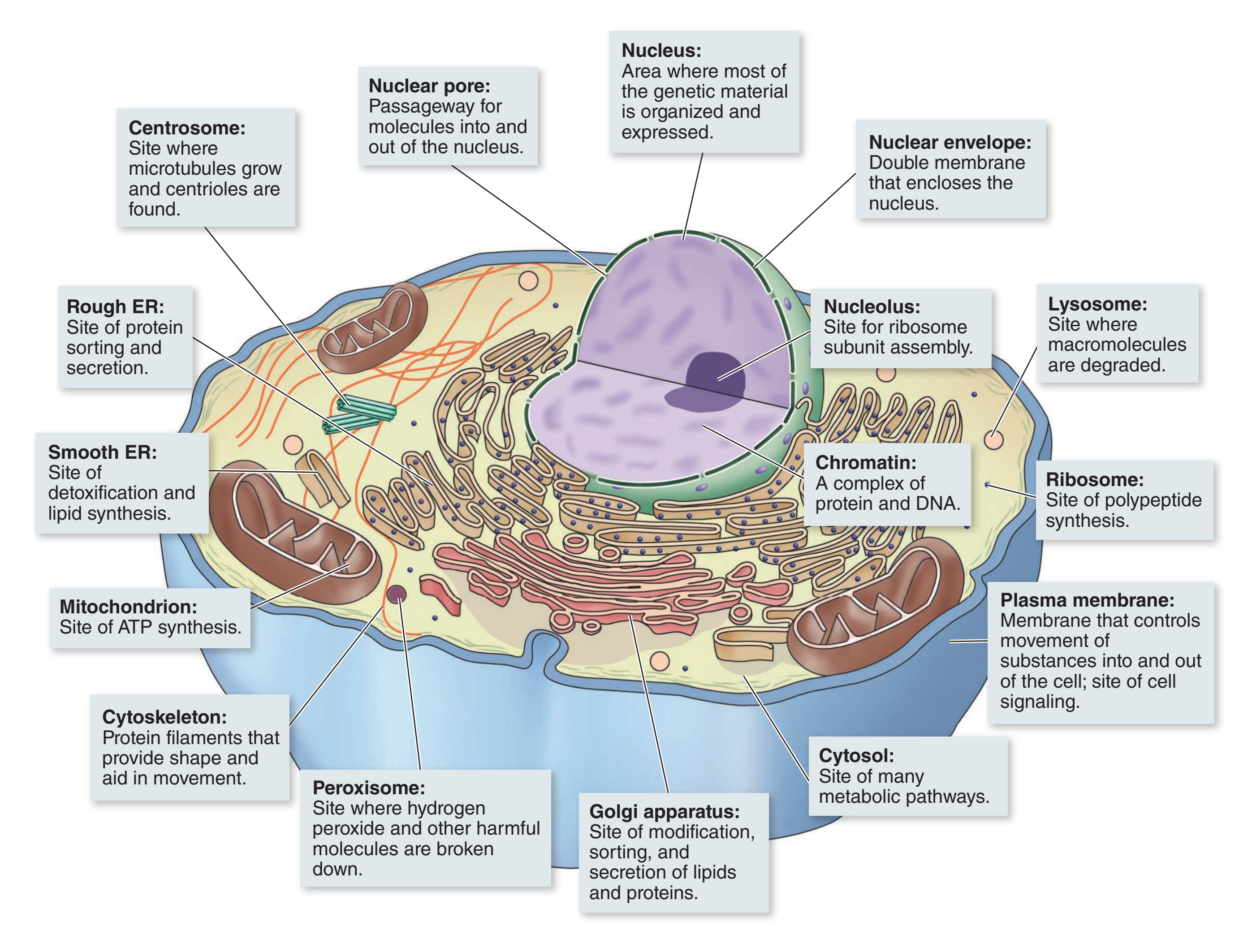


Figure 4.4 Structure of animal cells. Cells are surrounded by a bilayered plasma membrane containing phospholipids and proteins. The nucleus houses chromosomal DNA and is surrounded by a double-membraned nuclear envelope. Centrioles organize spindle fibers during cell division. Endoplasmic reticulum (ER) is a system of membranes inside the cell. Rough ER has many ribosomes, and smooth ER has fewer ribosomes. Mitochrondria are sites of oxidative respiration and ATP synthesis. Microvilli are cytoplasmic projections that increase the surface area of some specialized animal cells. Golgi complexes are flat sacs and vesicles that collect and package substances made in the cell. Ribosomes are aggregations of proteins that conduct protein synthesis. Lysosomes contain enzymes important in recycling cellular debris.

Question 4

a. What shape are the chloroplasts? What is their function?

b. Where are the chloroplasts located within the *Elodea* cell—toward the perimeter or center of the cell?

5. Determine the spatial distribution of chloroplasts within a cell. They may be pushed against the margins of the cell by the large **central vacuole** containing mostly water and bounded by a **vacuolar membrane.** The vacuole occupies about 90% of the volume of a mature cell. Its many functions include storage of organic and inorganic molecules, ions, water, enzymes, and waste products.

6. Search for a **nucleus;** it may or may not be readily visible. Nuclei usually are appressed to the cell wall as a faint gray sphere the size of a chloroplast or larger. Staining the cells with a drop of iodine may enhance the nucleus. If your preparation is particularly good, a **nucleolus** may be visible as a dense spot in the nucleus.

7. Search for some cells that may appear pink due to water-soluble pigments called anthocyanins. These

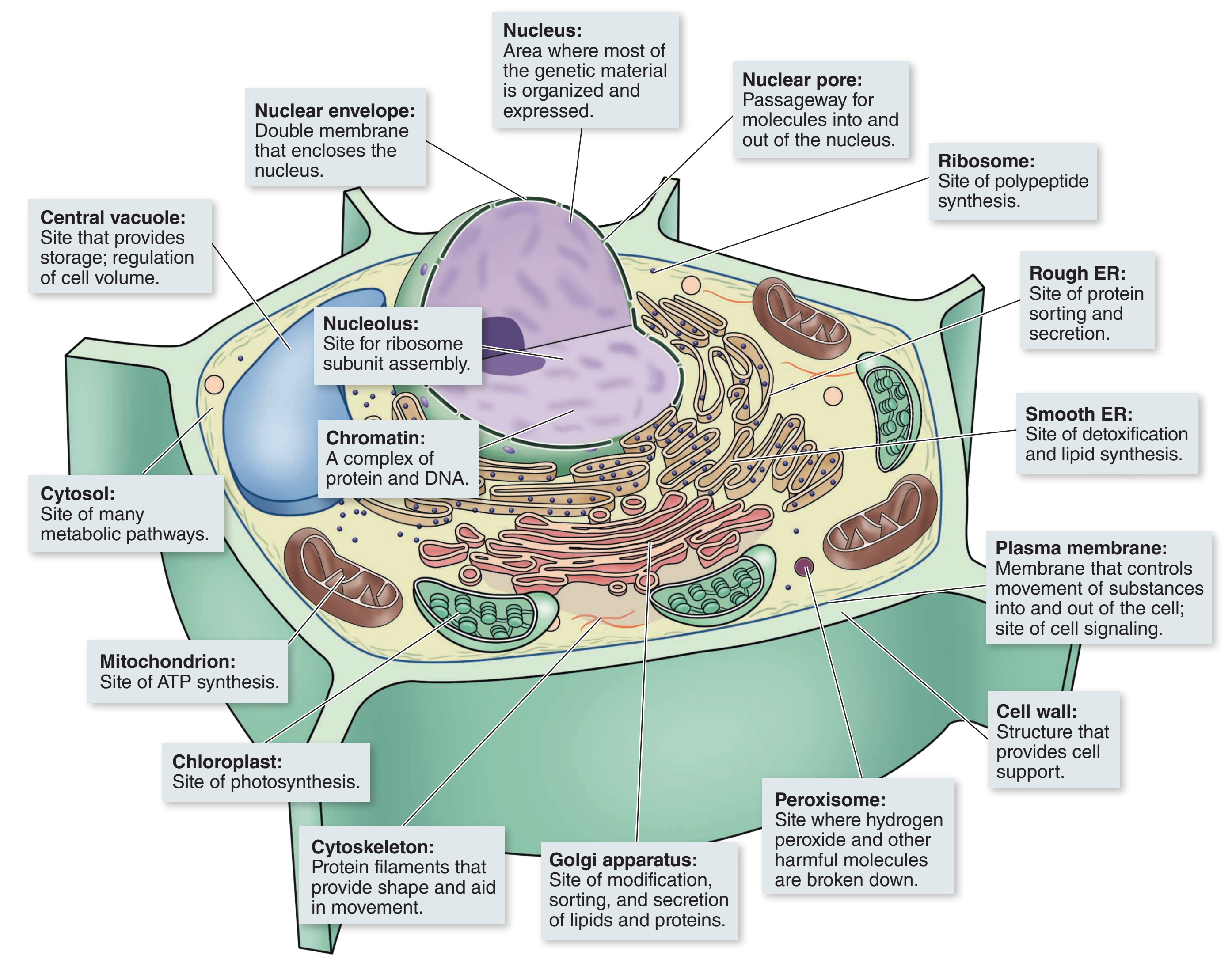


Figure 4.5 Structure of plant cells. Most mature plant cells contain large central vacuoles, which occupy most of the volume of the cell. Cytoplasm is often a thin layer between the vacuole and the plasma membrane. Cytoplasm contains the cell's organelles.

pigments give many flowers and fruits their bright reddish color.

8. Warm the slide with intense light for about 10 min and search for movement of the chloroplasts. You may need to search many cells or make a new preparation. This movement is called **cytoplasmic streaming,** or **cyclosis.** Chloroplasts are not motile; instead, they are being moved by the activity of the cytoplasm. Add water if the cells appear to be drying out.

9. In the following space sketch a few cells of *Elodea;* compare the cells with those shown in figure 4.6.

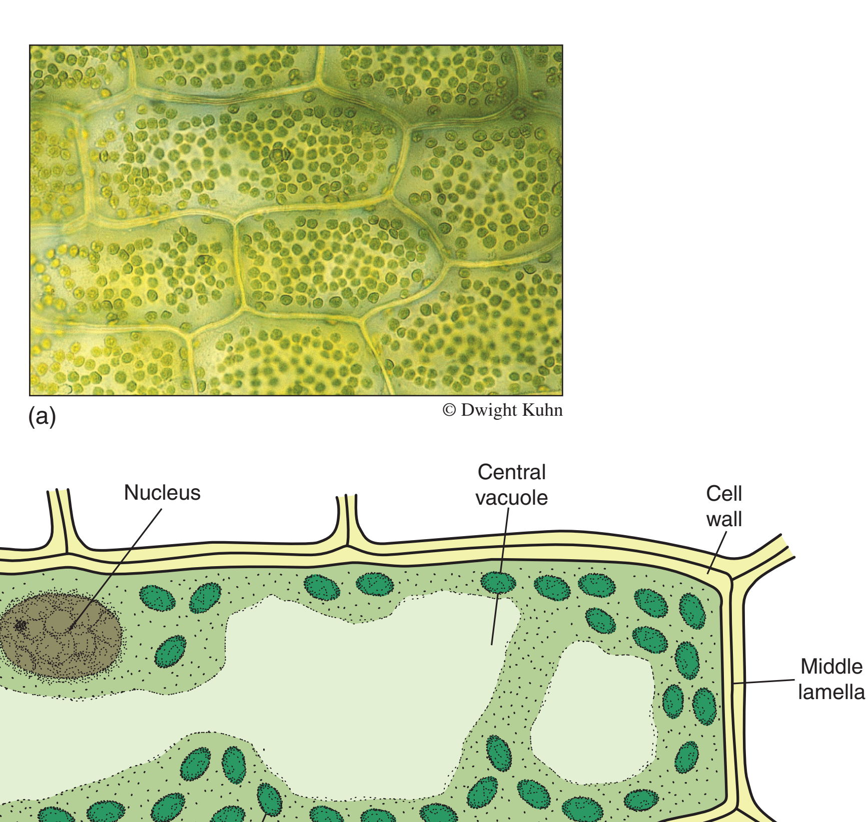
10. When you are finished examining *Elodea,* dispose of the *Elodea* as specified by your instructor.

Question 5

a. Can you see nuclei in *Elodea* cells?

b. What are the functions of nuclei?

Figure 4.6 (*a*) *Elodea* cells containing abundant chloroplasts (150×). (*b*) The cellular structure of *Elodea* (400×).



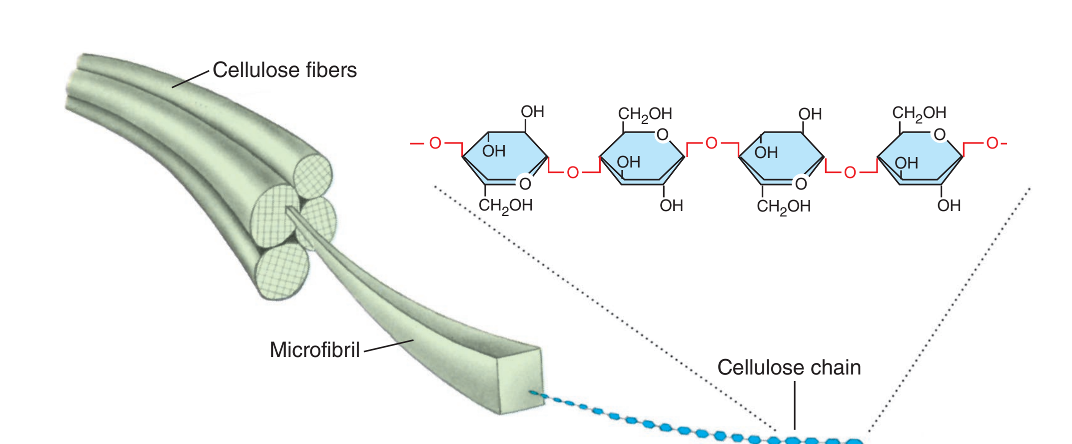
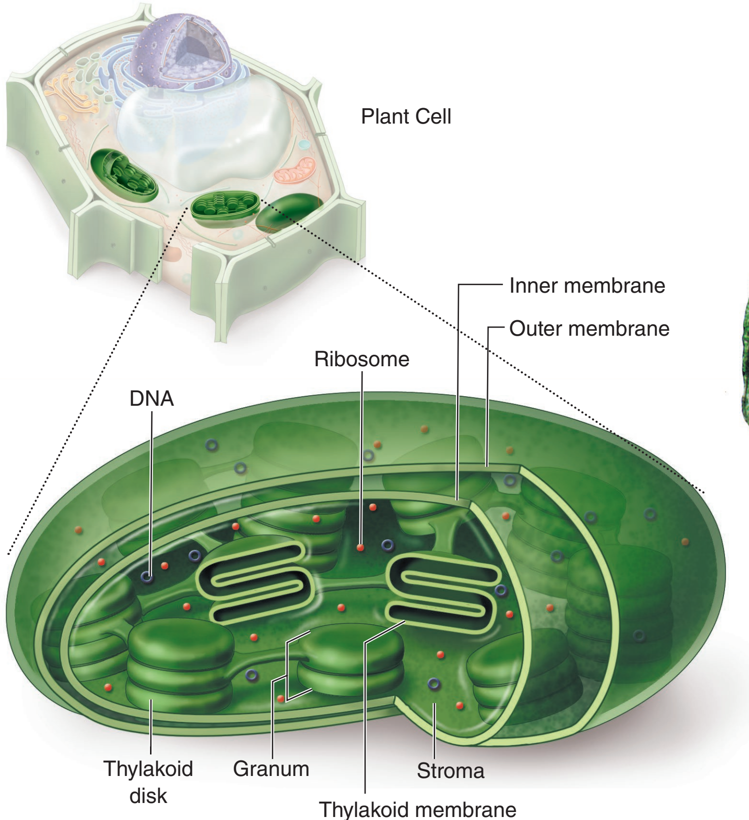


Figure 4.7 Cellulose is the most abundant organic compound on earth and is a polymer of glucose molecules. Free hydroxyl (OH^-) groups of the glucose molecules form hydrogen bonds between adjacent cellulose molecules to form cohesive microfibrils. Microfibrils align to form strong cellulose fibers that resist metabolic breakdown. Because humans cannot hydrolyze the bonds between glucose molecules of cellulose, cellulose is indigestible and its energy is unavailable. Cellulose passes through the human digestive tract as bulk fiber.



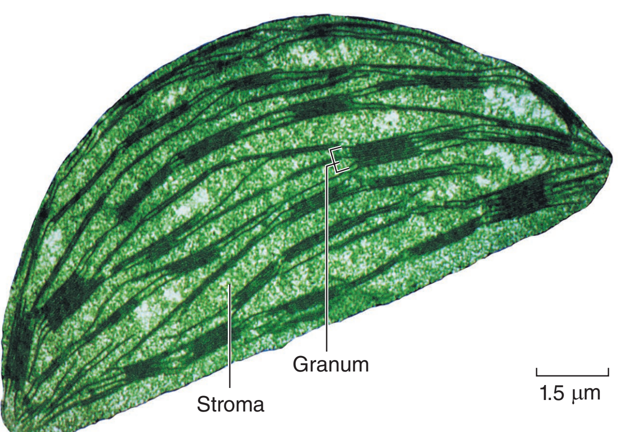


Figure 4.8 Chloroplast structure. The inner membrane of a chloroplast is fused to form stacks of closed vesicles called thylakoids. Photosynthesis occurs within these thylakoids. Thylakoids are typically stacked one on top of the other in columns called grana.

c. Which are larger, chloroplasts or nuclei?

d. What is the approximate size of a nucleus?

e. Why is the granular-appearing cytoplasm more apparent at the sides of a cell rather than in the middle?

Question 6

a. Are all cellular components moving in the same direction and rate during cytoplasmic streaming?

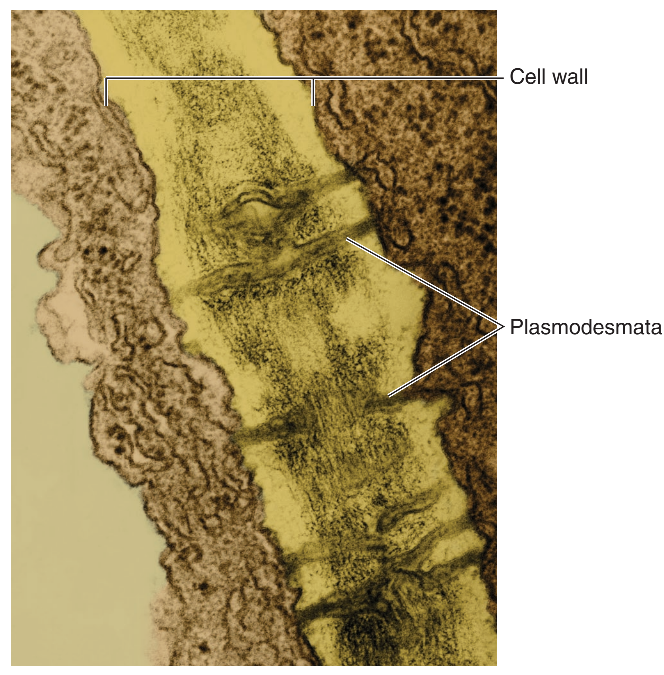
b. What do you conclude about the uniformity of cytoplasmic streaming?

Cell Walls

Cell walls include an outer **primary cell wall** deposited during growth of the cell and a **middle lamella,** the substance holding walls of two adjacent cells together. The protoplasm of adjacent cells is connected by cytoplasmic strands called **plasmodesmata** that penetrate the cell walls (fig. 4.9).

Procedure 4.4 **Examine cell walls and plasmodesmata**

1. Prepare a wet mount of *Elodea* and examine the cell walls. Always begin your examination at the lowest magnification and cautiously move to higher magnifications. The middle lamella may be visible as a faint line between cells.
2. Obtain a prepared slide of tissue showing plasmodesmata. This tissue may be persimmon (*Diospyros*) endosperm, which has highly thickened primary walls. Sketch what you see.



© Biophoto Associates/Science Source

Figure 4.9 This electron micrograph of the thickened primary cell walls of persimmon endosperm shows plasmodesmata connecting adjacent cells (130,000×).

3. Locate the middle lamella as a faint line between cell walls.
4. Locate the plasmodesmata appearing as darkened lines perpendicular to the middle lamella and connecting the protoplasts of adjacent cells (fig. 4.9).

Question 7

a. What are the functions of plasmodesmata?

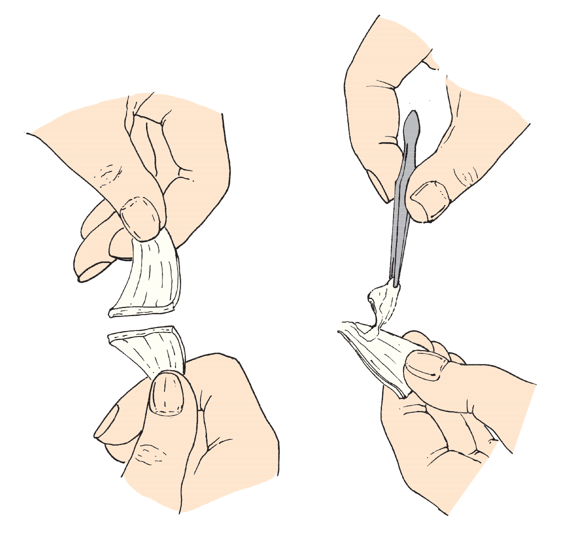
b. Why do you suspect that there are so many plasmodesmata connecting the cells in this fruit?

Onion Cells

Staining often reveals the structure of cells and cell organelles more clearly. A specimen is **stained** by adding a dye that preferentially colors some parts of the specimen but not others. Neutral red is a common stain that accumulates in the cytoplasm of the cell, leaving the cell walls clear. Nuclei appear as dense bodies in the translucent cytoplasm of the cells.

Procedure 4.5 Examine stained onion cells

1. Cut a red onion into eighths and remove a fleshy leaf.
2. Snap the leaf backward and remove the thin piece of the inner epidermis formed at the break point (fig. 4.10), as demonstrated by your lab instructor.
3. Place this epidermal tissue in a drop of water on a microscope slide, add a coverslip, and examine the tissue. This preparation should be one cell thick. Always begin your examination with the lowest magnification.
4. Stain the onion cells by placing a small drop of 0.1% neutral red at the edge of the coverslip. Draw the neutral red across the specimen by wicking. To wick the solution, hold the edge of a small piece of paper towel at the opposite edge of the coverslip and it will withdraw some fluid. This will cause the neutral red to flow over the onion and will not disturb the tissue under the coverslip.
5. Stain the tissue for 5–10 min.
6. Carefully focus to distinguish the vacuole surrounded by the stained cytoplasm.
7. Search for the nucleus of a cell (fig. 4.11). The nucleus may appear circular in the central part of the cell. In other cells it may appear flattened.



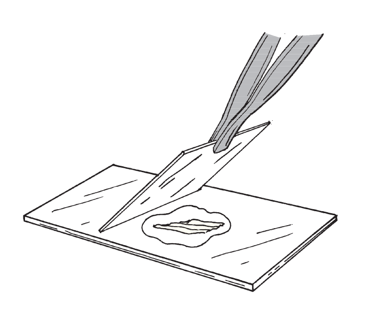


Figure 4.10 Preparing a wet mount of an onion epidermis.

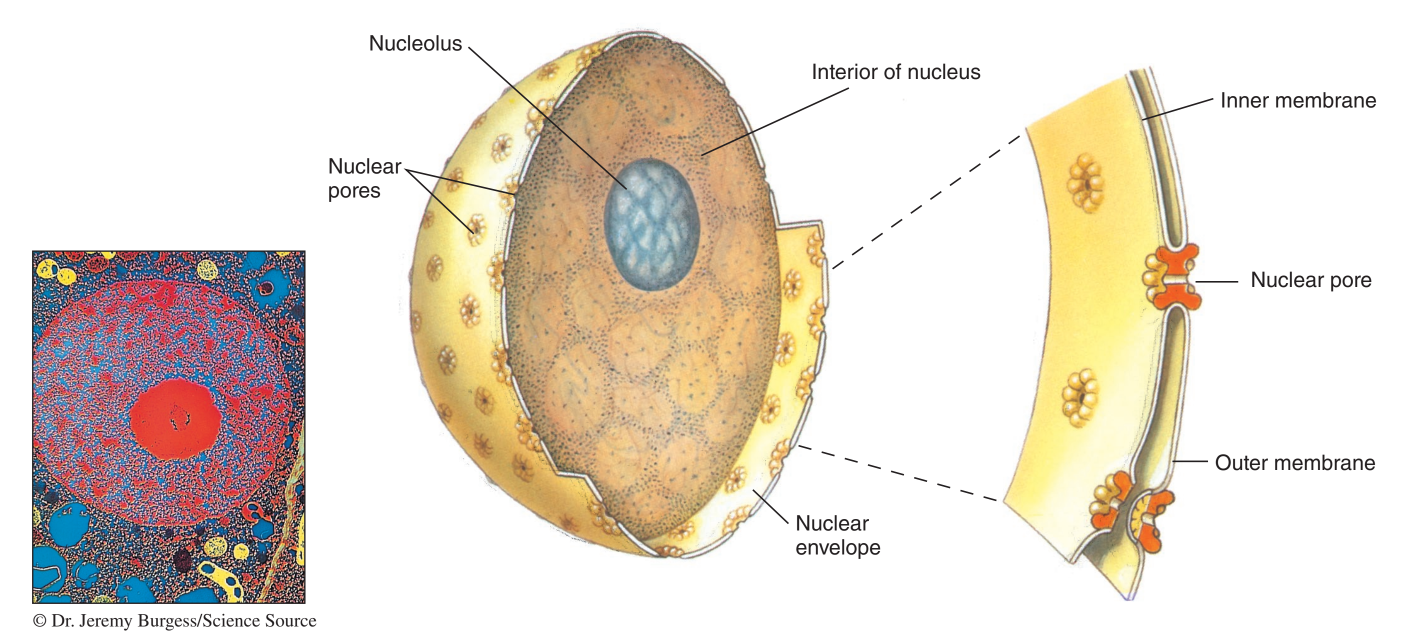


Figure 4.11 The nucleus. The nucleus consists of a double membrane, called a nuclear envelope, enclosing a fluid-filled interior containing the DNA. In the cross section, the individual nuclear pores extend through the two membrane layers of the nuclear envelope; the material within the pore is protein, which controls access through the pore (1765×).

Question 8
How do you explain the differences in the apparent shapes and positions of the nuclei in different cells?

8. Repeat steps 1–7 and stain a new preparation of onion cells with other available stains, such as methylene blue.
9. In the following space sketch a few of the stained onion cells.

Question 9
a. What cellular structures of onion are more easily seen in stained as compared to unstained preparations?

b. Which of the available stains enhanced your observations the most?

c. Do onion cells have chloroplasts? Explain.

d. Use an ocular micrometer or the dimensions of the field of view (FOV) calculated in Exercise 3 to measure the dimensions of an onion epidermal cell. Are these cells larger or smaller than the *Elodea* cells you examined in procedure 4.3?

Mitochondria

Mitochondria are surrounded by two membranes (fig 4.12). The inner membrane folds inward to form **cristae,** which hold respiratory enzymes and other large respiratory molecules in place. Some DNA also occurs in mitochondria. Chloroplasts also are double-membraned and contain DNA.

Procedure 4.6 Examine mitochondria in onion cells

1. On a clean glass slide mix two or three drops of the stain Janus Green B with one drop of 7% sucrose.
2. Prepare a thin piece of onion epidermis (as instructed in procedure 4.5) and mount it in the staining solution. The preparation should be one cell thick. For

mitochondria to stain well, the onion cells must be healthy and metabolically active. Add a coverslip.

3. Search the periphery of cells to locate stained mitochondria. They are small blue spheres about 1 μm in diameter. The color will fade in 5–10 min, so examine your sample quickly and make a new preparation if needed.

Plastids

Plastids are organelles where food, especially sugars and starch, is made and stored. You have already examined chloroplasts, a type of plastid in which photosynthesis occurs. Other plastids have different functions. We will examine **amyloplasts,** plastids that store starch and therefore will stain darkly with iodine.

Procedure 4.7 **Examine amyloplasts**

1. Use a razor blade to make a thin section of a potato tuber. Make the section as thin as you can.
2. Place the section in a drop of water on a microscope slide and add a coverslip. Add another drop of water to the edge if needed.
3. Locate the small, clam-shaped amyloplasts within the cells. High magnification may reveal the eccentric lines distinguishing layers of deposited starch on the grains.
4. Stain the section by adding a drop of iodine to the edge of the coverslip. Iodine is a stain specific for starch (see Exercise 6, "Biologically Important Molecules"). If necessary, pull the stain under the coverslip by touching a paper towel to the water at the opposite edge of the coverslip.

Question 10

a. Are any cellular structures other than amyloplasts stained intensely by iodine?

b. What can you conclude about the location of starch in storage cells of potato?

c. What are the functions of amyloplasts in potatoes?

d. Why are potatoes a good source of carbohydrates?

ANIMAL CELLS

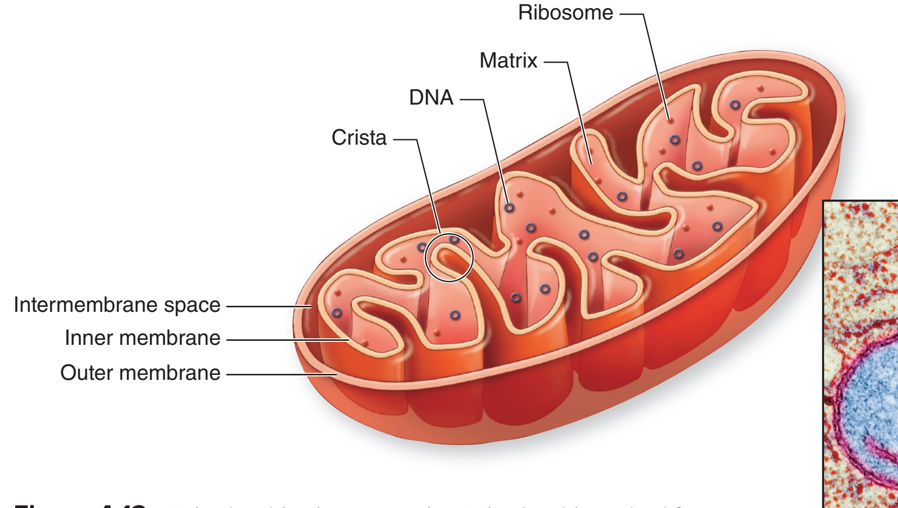
Animals, like plants, are eukaryotes. They share many similarities, and also have several differences (see table 4.1).

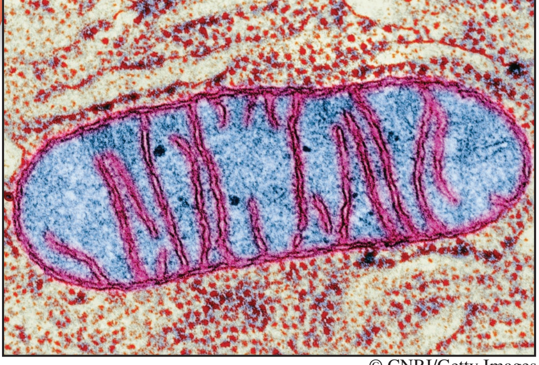
Human Epithelial Cells

Human epithelial cells are sloughed from the inner surface of your mouth. They are flat cells with a readily visible nucleus.

Procedure 4.8 **Examine human epithelial cells**

1. Gently scrape the inside of your cheek with the broad end of a clean toothpick.





© CNRI/Getty Images

Figure 4.12 Mitochondrion in cross section. Mitochondria evolved from bacteria that long ago took up residence within the ancestors of present-day eukaryotes (80,000×).

2. Stir the scrapings into a drop of water on a microscope slide, add a coverslip, and examine with your compound microscope. Dispose of used toothpicks in a container designated by your instructor.
3. Stain the cells by placing a small drop of methylene blue at one edge of the coverslip and drawing it under the coverslip with a piece of absorbent paper towel placed at the opposite side of the coverslip.
4. Prepare another slide and stain the cells with Janus Green B. Observe the mitochondria.
5. Use an ocular micrometer or the dimensions of the FOV calculated in Exercise 3 to measure the dimensions of a human epithelial cell.

Question 11

a. What structures visible in the stained preparation were invisible in the unstained preparation?

b. Were mitochondria as abundant in human epithelial cells as in onion epidermal cells (procedure 4.6)? Explain.

c. What similarities and differences are there between plant and animal cells?

d. How do the size and shape of a human epithelial cell differ from those of the *Elodea* and onion cells that you examined earlier?

e. Why do *Elodea* and onion cells have more consistent shapes than human epithelial cells?

6. After viewing the preparation, put the slides and coverslips in a container of 10% bleach.

PROTISTS

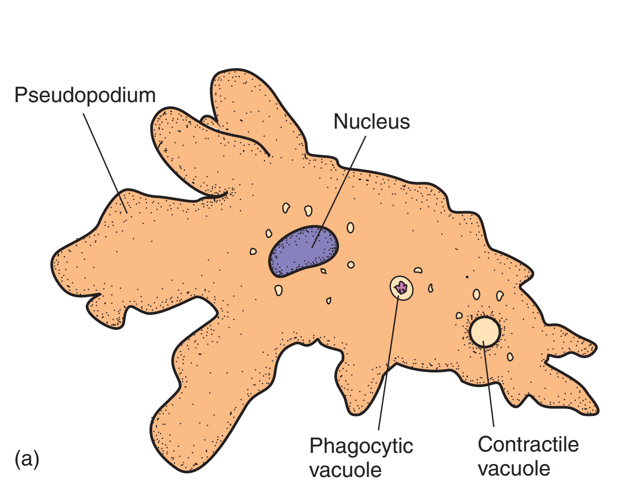
Amoeba, Paramecium, and *Spirogyra* are members of a large group of eukaryotic organisms called protists. You will learn more about protists in Exercises 25 and 26. In today's exercise, you'll examine *Amoeba, Paramecium,* and *Spirogyra*.

Amoeba

Amoeba is an irregularly shaped protist with many internal organelles (fig. 4.13). *Amoeba* move via amoeboid movement. **Amoeboid movement** occurs by means of **pseudopodia,** which are temporary protrusions of the cell. Pseudopodia also surround food particles and create food vacuoles, where food is digested. Another important structure in *Amoeba* is the **contractile vacuole** that accumulates and expels water and waste products.

Procedure 4.9 **Examine *Amoeba***

1. Use an eyedropper to obtain a few drops from the bottom of an *Amoeba* culture. Examining the culture with a dissecting microscope may help you locate some organisms.
2. Place the organisms on a microscope slide.
3. Add a coverslip and use a compound microscope to locate a living *Amoeba*. Your instructor may allow



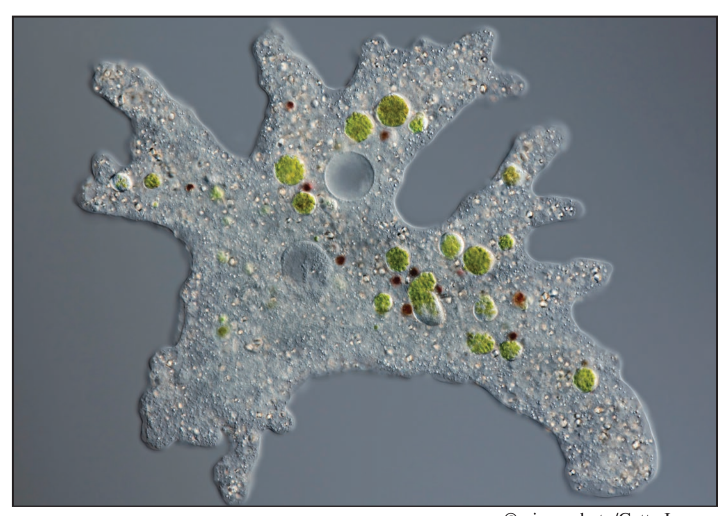


Figure 4.13 (*a*) Diagram of *Amoeba*. (*b*) Light micrograph of a living *Amoeba* (160×).

you to view the *Amoeba* without using a coverslip, but view them *only* on 4× or 10× magnification.

4. Decrease the light intensity and observe an *Amoeba* for a few minutes.
5. Locate the structures shown in figure 4.13.
6. Examine a prepared slide of stained *Amoeba;* then observe a demonstration of *Amoeba* on a dark-field microscope if one is available.
7. Sketch an *Amoeba* in the following space.

Question 12

a. List the organelles found in plant cells, in *Amoeba,* and common to both.

b. Does *Amoeba* have a cell wall? How can you tell?

c. How do the appearances of *Amoeba* differ in live cells and preserved cells?

Paramecium

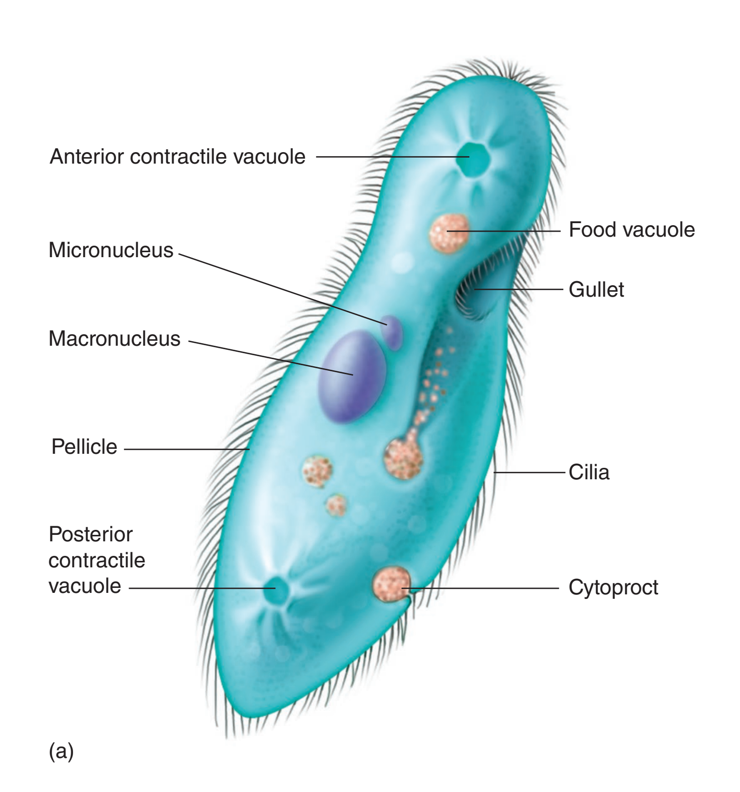
Like *Amoeba, Paramecium* is also a single-celled organism (fig. 4.14).

Procedure 4.10 Examine *Paramecium*

1. Place a small ring of methylcellulose on a microscope slide to slow the *Paramecium*.
2. Place a drop from a culture containing *Paramecium* inside the methylcellulose ring.
3. Use a toothpick to mix the methylcellulose with the drop of water from the culture of *Paramecium*.
4. Add a coverslip and examine *Paramecium* with your compound microscope. On the surface of *Paramecium* are cilia, which are short hairlike structures used for locomotion.
5. Examine a prepared slide of stained *Paramecium*.
6. In the following space, sketch a *Paramecium*.

Question 13

a. How does movement of *Paramecium* compare to that of *Amoeba?*



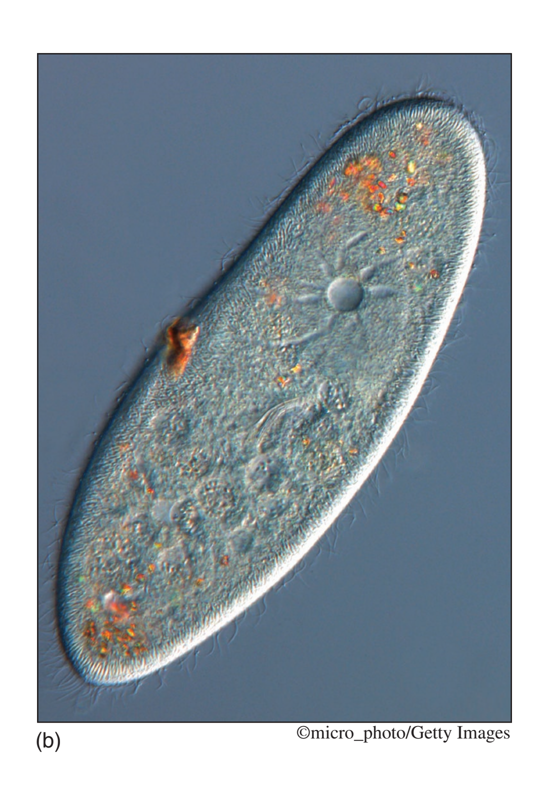


Figure 4.14 (*a*) Diagram of *Paramecium* (150×). (*b*) Light micrograph of a living *Paramecium*. Note the abundant cilia (150×).

b. How do shape and body consistency differ between *Amoeba* and *Paramecium?*

c. What structures in *Amoeba* and *Paramecium* also occur in plant cells? What structures in *Amoeba* and *Paramecium* do not occur in plant cells?

Spirogyra

Spirogyra (fig. 4.15) is a filamentous green alga that is named for the spiral arrangement of its chloroplasts. *Spirogyra* is common in freshwater ponds and streams, where it is a major part of "pond scum."

Procedure 4.11 Examine *Spirogyra*

1. Place a drop from a culture containing *Spirogyra* on a microscope slide.
2. Add a coverslip and examine *Spirogyra* with your compound microscope.
3. Sketch *Spirogyra* in the space below.



Question 14

a. Is *Spirogyra* branched or unbranched?

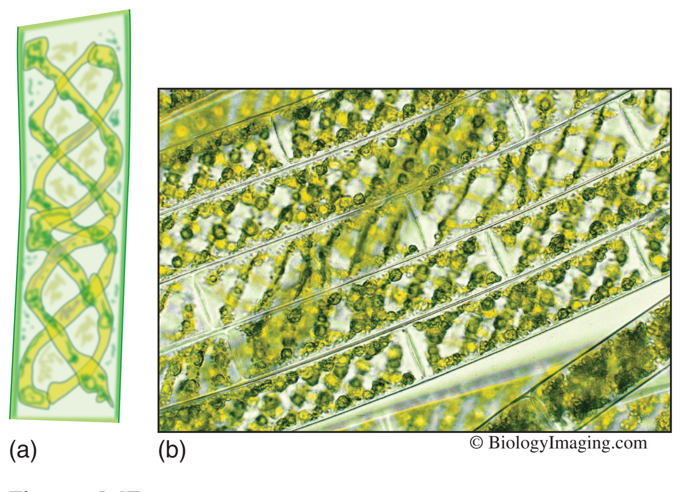


Figure 4.15 (*a*) Diagram of a *Spirogyra* cell (250×). (*b*) Light micrograph of a living *Spirogyra*. Note the spiral-shaped chloroplast for which the alga is named (200×).

b. In what shapes are the cells?

c. Do the cells have a cell wall? If so, how can you tell?

d. What organelles visible in *Spirogyra* are not visible in *Amoeba* and *Paramecium?*

Procedure 4.12

You will be given a slide of an unknown organism. Use what you've learned in today's lab to identify the cells as prokaryotic or eukaryotic; if eukaryotic, identify the cells as plant, animal, or protist. Complete table 4.2 before leaving the lab. If instructed to do so, turn in table 4.2 before leaving the lab.

INVESTIGATION

The Responses of Single-Celled Organisms to Environmental Stimuli

Observation: Single-celled protists such as *Paramecium* and *Amoeba* live in water and are sensitive to environmental stimuli.

Question: How are the movements of single-celled protists affected by temperature?

a. Establish a working lab group and obtain Investigation Worksheet 4 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 4 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Table 4.2

Using Distinguishing Features to Identify an Unknown Organism

Overall description of specimen:	Name ______________________ Unknown no: ______________ Lab section: ______________

Based on the above, my unknown organism is a: (Circle One)	Prokaryote	Eukaryote	
If the specimen is a eukaryote, it is a(n): (Circle One)	Plant	Animal	Protist

Questions for Further Thought and Study

1. What is a cell?

2. Describe the structure and function of each cellular part that you observed in this lab.

3. Would you expect a cell of a multicellular organism to be more complex than the cell of a unicellular organism? Less complex? Why?

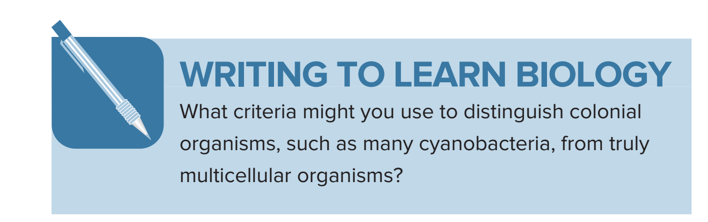
4. What is the purpose of using a biological stain when microscopically examining cellular components?

5. How are eukaryotic cells different from prokaryotic cells? How are they similar?

6. *Amoeba, Paramecuim,* and *Spirogyra* are diverse. Why, then, are they all classified as protists?

DOING BIOLOGY YOURSELF

Determine the total surface areas and volumes of the chloroplasts in a typical *Elodea* cell. Assume that each chloroplast is a sphere of 5 μm diameter. (The surface area of a sphere = πd^2; the volume of a sphere = $(\frac{4}{3})\pi r^3$.) What is the significance of these surface areas and volumes? Would it be advantageous for a cell to be filled with chloroplasts? Why or why not?



LEARNSMART LABS BIOLOGY

Exercise 5

Solutions, Acids, and Bases

The pH Scale

Learning Objectives

By the end of this exercise you should be able to:

1. Apply the concepts of mole and molarity to prepare solutions.
2. Measure the pH of various liquids.
3. Demonstrate that buffers stabilize the pH of a liquid.
4. Measure the ability of commercial antacids to buffer the pH of a liquid.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Chemicals in living systems are in solution. Biologists experiment with solutions because dissolved chemicals react more readily than solid, crystalline chemicals. A **solution** consists of a **solute**(s) dissolved in a **solvent.** For example, salt water is a solution in which salt (i.e., the solute) is dissolved in water (i.e., the solvent).

The concentration of a solute is often expressed as a percentage of the total solution (e.g., weight/volume or grams solute/100 mL solution). For example, a 3% (weight/volume) solution of sucrose is prepared by dissolving 30 g of sucrose in water for a total solution volume of 1 L (or 3 g of sucrose in water for a total volume of 100 mL).

Question 1

a. How many grams of sucrose would you dissolve in water for a total volume of 500 mL to make a 5% (weight/volume) solution?

b. How many grams of calcium chloride would you add to water for a total volume of 500 mL to make a 5% (weight/volume) solution?

c. How many grams of calcium chloride would you add to water for a total volume of 100 mL to make a 5% (weight/volume) solution?

Molarity is the most common measure of concentration. To understand how to prepare a molar solution you must first understand what is meant by a **mole** of a chemical. A mole is a standard measure of the amount of a chemical—one mole of any substance has 6.02×10^{23} molecules (Avogadro's number). One mole of NaCl and one mole of sucrose contain the same number of molecules. However, a mole of NaCl and a mole of sucrose weigh different amounts. This is because each chemical has a different **molecular weight.** The weight of 1 mole of a chemical equals that chemical's molecular weight in grams. For example, the molecular weight of water (H_2O) is 18 g ($2H = 2 \times 1 = 2$; $O = 16$; $16 + 2 = 18$). A mole of water weighs 18 g. A mole of NaCl weighs 58.5 g (fig. 5.1). A chemical's molecular weight is the sum of the atomic weights of its component elements.

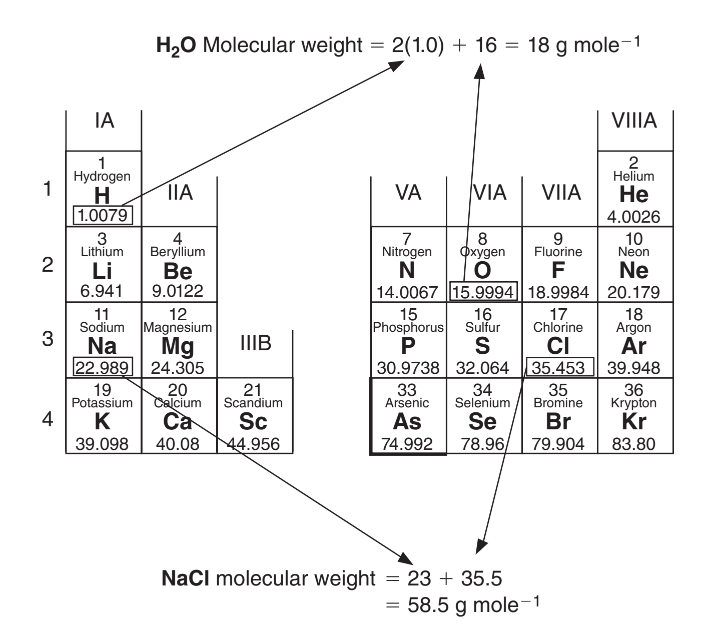


Figure 5.1 The atomic weights of elements are listed in the periodic table. Shown here are the portions of the periodic table that would be used to calculate the molecular weights of water (H_2O) and table salt (sodium chloride, NaCl). Note that g mole^{-1} = grams per mole.

To further understand why biologists usually prepare solutions in molar concentrations rather than as percentages you must remember that chemicals react on a molecule by molecule basis—that is, the number of molecules is more critical than the weight. It follows that expressing a solution's concentration in moles is a better measure of how much chemical is available to react. A solution that contains one mole of a chemical in 1 liter of solution has 6.02×10^{23} molecules available and is a 1-molar (1 M) solution. For example, a liter of solution containing 58.5 g of NaCl is a 1 M solution of NaCl (fig. 5.2).

To ensure that you have some practice with making basic chemical calculations, answer Questions 2 and 3 before coming to lab. Your instructor may want to check your answers during the lab period.

Question 2

a. How many grams of NaCl (molecular weight = 58.5 g $mole^{-1}$) would you dissolve in water to make a 0.5 M NaCl solution with 500 mL final volume?

_________ g

b. How many grams of NaCl (molecular weight = 58.5 g $mole^{-1}$) would you dissolve in water to make a 50 mM NaCl solution with 500 mL final volume?

_________ g

c. How many grams of sucrose (molecular weight = 342 g $mole^{-1}$) would you dissolve in water to make a 0.22 M sucrose solution with 1 L final volume?

_________ g

d. How many grams of sucrose (molecular weight = 342 g $mole^{-1}$) would you dissolve in water to make a 0.22 mM sucrose solution with 100 mL final volume?

_________ g

e. How many grams of calcium chloride ($CaCl_2$; molecular weight = 111 g $mole^{-1}$) would you dissolve in water to make a 0.111 M $CaCl_2$ solution with 1 L final volume?

_________ g

f. How many grams of calcium chloride ($CaCl_2$; molecular weight = 111 g $mole^{-1}$) would you dissolve in water to make a 0.2 M $CaCl_2$ solution with 200 mL final volume?

_________ g

g. If you were presented with 2 L of a 2 M sucrose stock solution, how many grams of sugar would be in a 100 mL aliquot?



_________ g

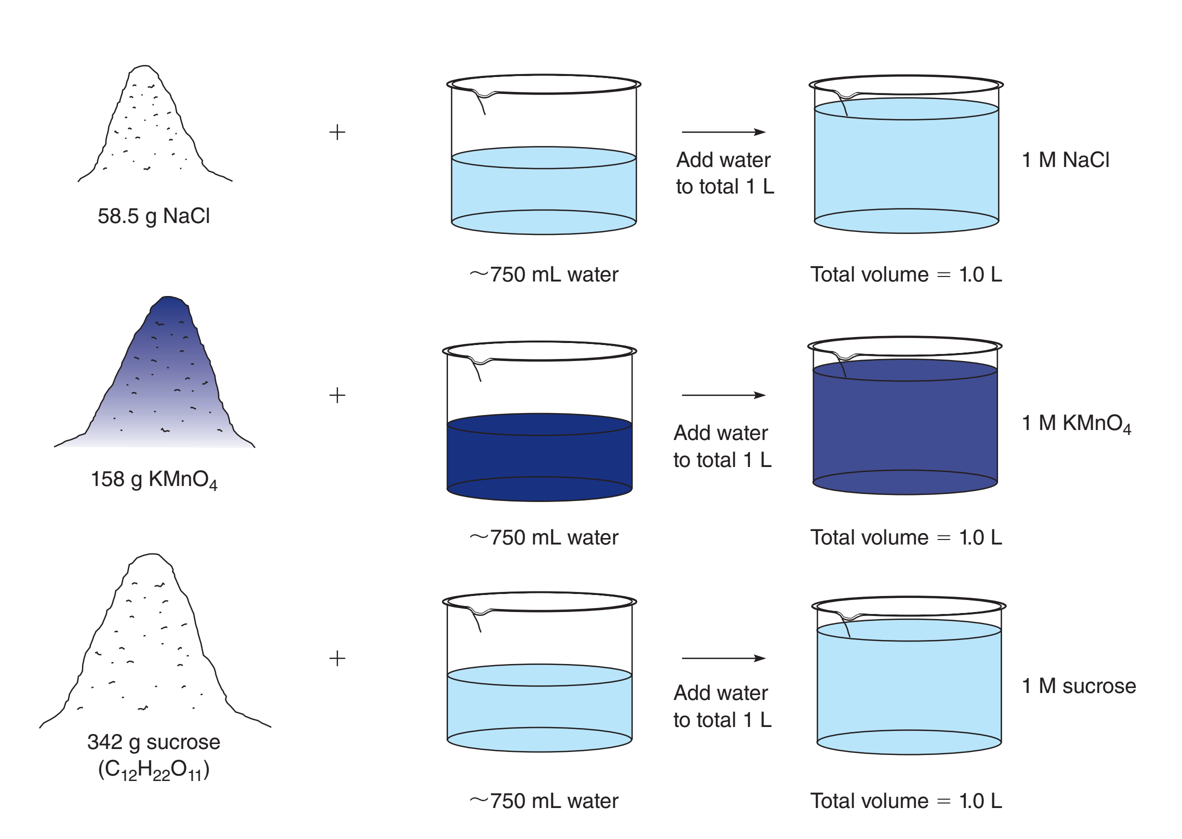


Figure 5.2 Preparing 1.0 M solutions of sodium chloride (NaCl; molecular weight = 58.5 g $mole^{-1}$), potassium permanganate ($KMnO_4$; molecular weight = 158 g $mole^{-1}$), and sucrose ($C_{12}H_{22}O_{11}$; molecular weight = 342 g $mole^{-1}$). Each of these solutions contains the same number of units of solutes (i.e., 6.02×10^{23} molecules).

h. To prepare the 5% sucrose solution called for in Question 1a, how many moles of sugar did you add? What was the molarity of that solution?

i. To prepare the 5% calcium chloride solution called for in Question 1b, how many moles of calcium chloride did you add? What was the molarity of that solution?

j. How many milliliters of a 2 M sucrose solution would contain 1 mole of sucrose?

Dilutions

To save time and space, biologists often prepare commonly used solutions in concentrated forms called **stock solutions.** These stock solutions are then diluted with water to make new solutions having a desired molarity. This process is called **dilution.**

Dilution involves spreading a given amount of solute throughout a larger solution. The number of moles of solute doesn't change when a solution is diluted but the volume of solution containing those moles increases. This means that the product of the initial volume (V_i) and initial molarity (M_i) must equal the product of the final volume (V_f) and final molarity (M_f):

$$V_i M_i = V_f M_f$$

where

$$V_i = \text{initial volume}$$
$$M_i = \text{initial molarity}$$
$$V_f = \text{final volume}$$
$$M_f = \text{final molarity}$$

Let's now use this simple equation to solve a dilution problem. Suppose we want to know how much water to add to 25 mL of a 0.50 M KOH solution to produce a solution having a KOH concentration of 0.35 M. In this case,

$$M_i = 0.5 \text{ M}$$
$$V_i = 25 \text{ mL}$$
$$M_f = 0.35 \text{ M}$$
$$V_f = ?$$

We can now solve the problem:

$$V_i M_i = V_f M_f$$
$$(25 \text{ mL})(0.5 \text{ M}) = (V_f)(0.35 \text{ M})$$
$$V_f = 35.7 \text{ mL}$$

The initial volume (V_i) was 25 mL, so we must subtract 25 mL from 35.7 mL to get our answer: 35.7 mL − 25 mL = 10.7 mL of water to produce a KOH solution having a concentration of 0.35 M.

Question 3

a. How many milliliters of concentrated (18 M) sulfuric acid (H_2SO_4) are required to prepare 750 mL of 3 M sulfuric acid?

b. How would you prepare 100 mL of 0.4 M $MgSO_4$ from a stock solution of 2 M $MgSO_4$?

c. How many milliliters of water would you add to 100 mL of 1.0 M HCl to prepare a final solution of 0.25 M HCl?

ACIDS AND BASES

One of the most important applications of molarity involves the concentration of hydrogen ions (H^+) in a solution. Pure water is the standard by which all other solutions are compared, because pure water is an ionically neutral solution. This neutrality is not due to the absence of ions, but rather to the equal concentrations of positive and negative ions. When the oxygen of water pulls hard enough on an electron from one of its hydrogens, two ions form:

$$H_2O \leftrightarrow H^+ + OH^-$$

This dissociation of water is rare and reversible, but it happens often enough for the concentration of H^+ in pure water to be 10^{-7} M. The solution is neutral because the concentration of OH^- is also 10^{-7} M. The sum of H^+ and OH^- ions will always equal 10^{-14}.

Acids are molecules that release hydrogen ions (H^+) when dissolved in water. Acids increase the concentration of H^+ in a solution. **Bases** are molecules that remove H^+ from solution. Bases decrease the concentration of H^+ in a solution. When the concentration of H^+ increases, the concentration of OH^- becomes proportionately less. For example, hydrochloric acid (HCl) quickly ionizes in water and increases the concentration of H^+; therefore, HCl is an acid. In contrast, sodium hydroxide (NaOH) is a base because it ionizes and increases the concentration of OH^-, thereby lowering the relative proportion of H^+. Thus, if enough acid

is added to water to raise the H^+ concentration to 10^{-6} M, the OH^- concentration would decrease to 10^{-8} M.

By general agreement, the scale we use to measure acidity is the **pH scale** (*pH* stands for the potential of hydrogen ions). The pH is the negative logarithm of the concentration of H^+; that is,

$$\mathbf{pH = -log\ [H^+]}$$

As pH goes up, the concentration of H^+ goes down. (The brackets indicate concentration of hydrogen ions.) The pH scale ranges from 0 ($-\log 10^{\infty}$; most acidic) to 14 ($-\log 10^{-14}$; most basic). On this scale, pure water has a pH of 7 ($-\log 10^{-7}$); pH values less than 7 are acidic, whereas those above 7 are basic (fig. 5.3).

Figure 5.3 shows the pHs of some common (and a few not-so-common) substances. The pH scale is a logarithmic scale; each unit represents a change of tenfold. Thus, a lime with a pH of 2 is ten times more acidic than an apple with a pH of 3, and 100 times more acidic than a tomato having a pH of 4. Each decrease of 1.0 pH unit represents a tenfold increase in acidity. Each increase of 1.0 pH unit represents a tenfold decrease in acidity.

Question 4

a. Vinegar has a pH of 3, and household ammonia has a pH of 11. Is the concentration of H^+ greatest in the vinegar or ammonia?

b. How many times different is the concentration?

Measuring pH

A convenient way of measuring the pH of a solution is with **pH paper.** pH paper is treated with a chemical indicator that changes colors depending on the concentration of H^+ in

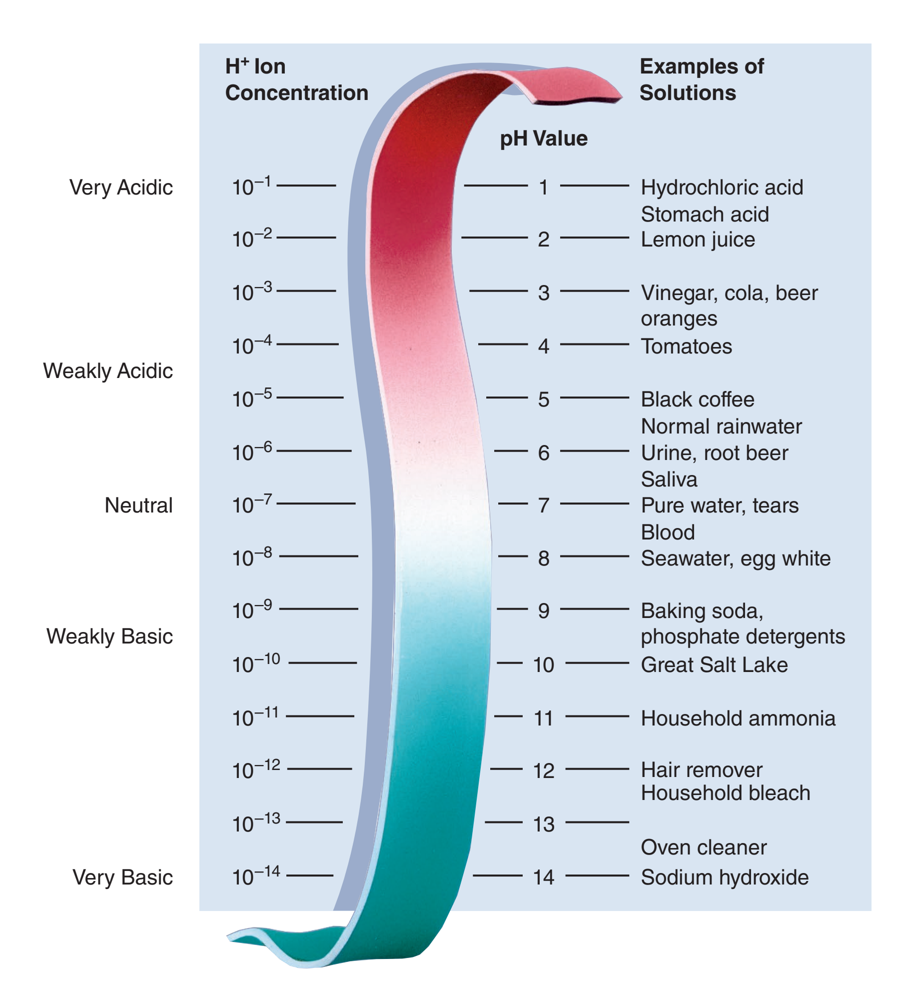


Figure 5.3 The pH scale. The pH value of a solution indicates its concentration of hydrogen ions. Solutions with a pH less than 7 are acidic, whereas those with a pH greater than 7 are basic. The pH scale is logarithmic: a pH change of 1 means a tenfold change in the concentration of hydrogen ions. Thus, lemon juice is 100 times more acidic than tomato juice, and seawater is 10 times more basic than pure water, which has pH of 7.



Figure 5.4 Indicator pH paper is embedded with chemicals that change color according to the pH of a solution. According to the color chart provided on the container of pH paper, the lemon juice sampled with the paper strip on the left has a pH of 2. The pH test strip on the right indicates that the sodium hydroxide solution has a pH of 12.

the solution that it has contacted (fig. 5.4). The color chart on the container of pH paper relates the color of the pH paper to the pH of the solution. Here are some examples of pH indicators:

Indicator	*Range*	*Color Change*
Methyl violet	0.2–3.0	yellow to blue-violet
Bromophenol blue	3.0–4.6	yellow to blue
Methyl red	4.4–6.2	red to yellow
Litmus	4.5–8.3	red to blue
Bromcresol purple	5.2–6.8	yellow to purple
Phenol red	6.8–8.0	yellow to red
Thymol blue	8.0–9.6	yellow to blue
Phenolphthalein	8.3–10.0	colorless to red



SAFETY FIRST Before coming to lab you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.



Handle all of the solutions carefully. Although some are harmless (e.g., water, milk), others are caustic and can stain clothes and burn your skin.

Procedure 5.1 Measure the pH of liquids

Use pH papers to measure the pH of the following liquids. Be as accurate as possible and use a fresh piece of pH paper or pH dipstick for each test.

Vinegar	________
Skim milk	________
Apple juice	________
Grapefruit juice	________
Buttermilk	________
Black coffee	________
Sprite	________
Household bleach	________
Mixture of Sprite and baking soda	________
10 mM hydrochloric acid	________
1.0 mM hydrochloric acid	________
0.01 mM hydrochloric acid	________
Distilled water	________
Tap water	________
Dissolved aspirin	________
Soap solution	________
Shampoo	________
Mouthwash	________
Deodorant	________

Check your measurements of the hydrochloric acid solutions by comparing them with calculations using the following formula. For example,

$$\mathbf{pH = -\log[H^+]}$$
$$\mathbf{10\ mM\ HCl = 10^{-2}\ M\ HCl}$$
$$\mathbf{pH = -\log[10^{-2}]}$$
$$\mathbf{pH = 2}$$

Question 5

Are your measured pH values similar to the calculated pH values? What are possible sources of error?

Buffers

In most organisms, the pH is kept relatively constant by **buffers,** which are mixtures of a weak acid and a weak base that can combine with a strong acid or base to limit changes in pH. That is, buffers absorb excess H^+ as the pH decreases or release H^+ as the pH increases. Buffers minimize changes in pH (fig. 5.5). The addition of a small amount of acid to a buffered solution produces a small change in pH, whereas adding the same amount of acid to an unbuffered solution changes the pH drastically. Most biological fluids

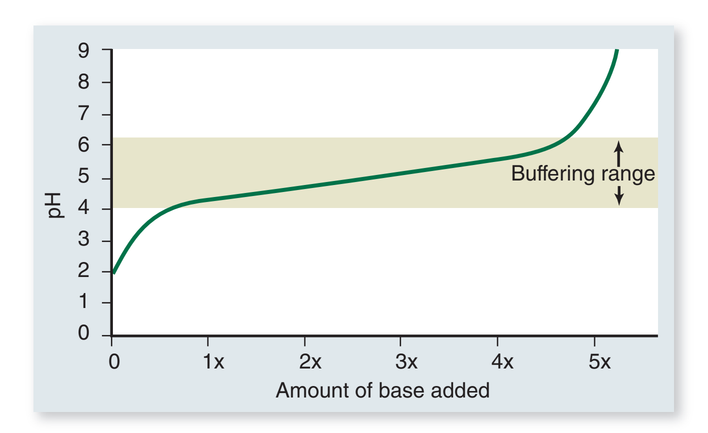


Figure 5.5 Buffers minimize changes in pH. Adding a base to a solution will raise the pH (neutralize some of the acid present). Thus, as more and more base is added, the pH continues to rise. However, a buffer makes the curve rise or fall very slowly over a portion of the pH scale, called the "buffering range" of that buffer.

(e.g., milk, blood) contain buffers, the most important of which is bicarbonate:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

For example, human blood contains buffers that maintain a pH of 7.3–7.5; blood pH above 7.5 produces alkalosis, and blood pH below 7.3 produces acidosis. Both of these conditions can cause illness and even death.

Procedure 5.2 Test the ability of buffers to stabilize pH

1. Obtain and label four test tubes to receive the four solutions listed in table 5.1.
2. Place 5 mL of each solution into its appropriately labeled tube.
3. Measure the pH of each of the solutions in the tubes and record these initial values in table 5.1.
4. Add 5 drops of acid (0.1 M HCl) to the first tube. Cover the tube with Parafilm and swirl the tube gently to mix the contents.
5. Measure the pH of the acidified solution and record it in table 5.1.
6. Repeat steps 4 and 5 for each of the remaining tubes. Record your results in table 5.1.

Question 6

a. Compare the initial pH and the pH after adding acid to each sample. Which is the most effective buffer? Which is least effective?

b. What accounts for the different buffering capacities of these fluids?

c. What is the biological importance of what you observed?

Procedure 5.3 Test the effectiveness of commercial antacids and other products

Commercial antacids such as *Alka-Seltzer, Rolaids,* and *Tums* claim to "neutralize stomach acid" by absorbing excess H^+ (produced as hydrochloric acid by the stomach; fig 5.6). To test the abilities of these products to absorb acids, do the following:

1. Use a mortar and pestle to pulverize the amount of antacid that is listed as one dose. Dissolve the crushed antacid in 100 mL of distilled water. Some of the products may require extensive stirring to get most or all of the powder to dissolve.
2. Use a pipet or 10-mL graduated cylinder to add 5 mL of the antacid solution into a test tube. Add 4 drops of the indicator bromcresol purple to the tube. Cover the tube with Parafilm and invert the tube to mix the contents.
3. Add 0.1 M hydrochloric acid (HCl) dropwise to the tube; mix after each drop. Continue this process until the solution turns yellow, indicating an acidic solution.

Table 5.1

Testing the Buffering Capacity of Various Solutions

Procedure 5.2 Solution	Initial pH	pH after Adding Acid	Procedure 5.3 Solution	Drops of Acid
Water	________	________	*Alka-Seltzer*	________
0.1 M NaCl	________	________	*Rolaids*	________
Skim milk	________	________	*Tums*	________
0.1 M phosphate buffer	________	________		



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Figure 5.6 These commercially available products soothe or prevent acid indigestion, upset stomach, and related problems. How do the products shown here do this?



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Figure 5.7 Several over-the-counter products claim to reduce heartburn and acid indigestion. How do the products shown here do this?

4. Record in table 5.1 the number of drops of acid needed to generate the change of color. This number of drops is an index to the amount of acid (H^+) that the solution neutralizes before the pH drops below the yellow endpoint of bromcresol purple (pH 5.2).

Question 7

a. Which antacid neutralizes the most acid? Which neutralizes the least acid?

b. What is the effect of dose (for example, the size of tablets or the amount of antacid per tablet) on your results and conclusions?

c. Examine the packages of the products you tested. What are the active ingredients of each product? What does this tell you about how these products work?

Many people also use products such as *Zantac, Pepcid AC, Gaviscon, Prilosec, Tagamet, Pepcid AC Complete, Maalox,* and *Zantac 75* to soothe upset stomachs (fig. 5.7). Examine these products in lab, noting their claims and active ingredients. Based on your observations, write a hypothesis predicting each product's ability to absorb acid.

Now use procedure 5.3 to test each product's ability to absorb acid. List your results here.

Question 8

a. How accurate were your hypotheses?

b. How does each product work?

INVESTIGATION

The Properties of Phillips' Milk of Magnesia*, a Popular Antacid*

Observation: *Phillips' Milk of Magnesia* is a milky-white liquid that is a popular over-the-counter laxative and antacid. *Phillips' Milk of Magnesia* is often taken by people suffering from "acid indigestion."

Question: How effective is *Phillips' Milk of Magnesia* at neutralizing acid?

a. Establish a working lab group and obtain Investigation Worksheet 5 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 5 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your question, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What do buffers do and why are they important in biological systems?

2. Our stomachs secrete hydrochloric acid. Knowing the function of antacids, what do you think causes most "upset stomachs"?

3. The soft drink *Mr. Pibb* contains (among other things) 39 g of sucrose in 355 mL of solution. What is the molarity of this sucrose solution? What is the percentage (weight/volume) of sucrose in the solution?

4. Our stomachs secrete hydrochloric acid. What functions does this hydrochloric acid serve?

5. Suppose that the concentration of H^+ in Solution #1 is 10,000 times greater than Solution #2. What can you conclude about the difference in pH of these two solutions?

6. What is the active ingredient in *Phillips' Milk of Magnesia?* How is this different from that of products such as *Tums?*



Exercise 6

Biologically Important Molecules

Carbohydrates, Proteins, Lipids, and Nucleic Acids

Learning Objectives

By the end of this exercise you should be able to:

1. Perform tests to detect the presence of biologically important carbohydrates, proteins, lipids, and nucleic acids.
2. Explain the importance of a positive and a negative control in biochemical tests.
3. Use biochemical tests to identify an unknown compound.



Please visit connect.mheducation.com to review online resources tailored to this lab.

Most organic compounds in living organisms are **carbohydrates, proteins, lipids,** or **nucleic acids.** Each of these macromolecules is made of smaller subunits. These subunits are linked by **dehydration synthesis,** which is an energy-requiring process in which a molecule of water is removed and the two subunits are bonded covalently (fig. 6.1). Similarly, breaking the bond between the subunits requires the addition of a water molecule and releases energy. This energy-releasing process is called **hydrolysis.**

The subunits of macromolecules are held together by covalent bonds and have different structures and properties. For example, lipids (made of fatty acids) have many C—H bonds and relatively little oxygen, while proteins (made of amino acids) have amino groups (—NH_2) and carboxyl (—COOH) groups. These characteristic subunits and groups impart different chemical properties to macromolecules—for example, monosaccharides such as glucose are polar and soluble in water, whereas lipids are nonpolar and insoluble in water.

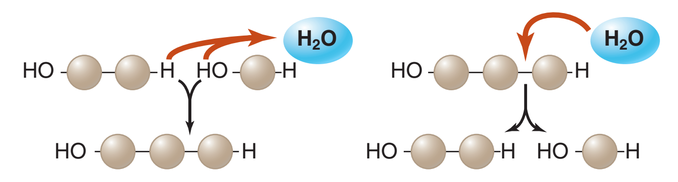


Figure 6.1 Making and breaking macromolecules.
(*a*) **Dehydration synthesis.** Biological macromolecules are polymers formed by linking subunits together. The covalent bond between the subunits is formed by dehydration synthesis, an energy-requiring process that creates a water molecule for every bond formed.
(*b*) **Hydrolysis.** Breaking the bond between subunits requires the returning of a water molecule with a subsequent release of energy, a process called hydrolysis.

CONTROLLED EXPERIMENTS TO IDENTIFY ORGANIC COMPOUNDS

Scientists have devised several biochemical tests to identify the major types of organic compounds in living organisms. Each of these tests involves two or more treatments: (1) an **unknown solution** to be identified, and (2) **controls** to provide standards for comparison. As its name implies, an unknown solution may or may not contain the substance that the investigator is trying to detect. Only a carefully conducted experiment will reveal its contents. In contrast, controls are known solutions. We use controls to validate that our procedure is detecting what we expect it to detect and nothing more. During the experiment we compare the unknown solution's response to the experimental procedure with the control's response to that same procedure.

A **positive control** contains the variable for which you are testing; it reacts positively and demonstrates the test's ability to detect what you expect. For example, if you are testing for protein in unknown solutions, then an appropriate positive control is a solution known to contain protein. A positive reaction shows that your test reacts as expected; it also shows you what a positive test looks like.

A **negative control** does not contain the variable for which you are searching. It contains only the solvent (often distilled water with no solute) and does not react in the test. A negative control shows you what a negative result looks like.

CARBOHYDRATES

Benedict's Test for Reducing Sugars

Carbohydrates are molecules made of C, H, and O in a ratio of 1:2:1 (e.g., the chemical formula for glucose is $C_6H_{12}O_6$). Carbohydrates are made of **monosaccharides,** or simple sugars (fig. 6.2). Paired monosaccharides form **disaccharides**—for example, sucrose (table sugar) is a

Glucose, a monosaccharide

Sucrose, a disaccharide made of glucose (left) and fructose (right)

Polysaccharides are polymers of monosaccharides

Figure 6.2 Carbohydrates consist of subunits of mono- or disaccharides. These subunits can be combined by dehydration synthesis (see fig. 6.4) to form polysaccharides.

disaccharide of glucose linked to fructose. Similarly, linking three or more monosaccharides forms a **polysaccharide** such as starch, glycogen, or cellulose (fig. 6.3).

Question 1

Examine figure 6.2. Which groups of a glucose molecule are involved in forming a polysaccharide? Shade the groups with a pencil.

As already mentioned, the linkage of subunits in carbohydrates, as well as other macromolecules, involves the removal of a water molecule (dehydration). Figure 6.4 depicts how dehydration synthesis is used to make maltose and sucrose, two common disaccharides.

Many monosaccharides such as glucose and fructose are **reducing sugars,** meaning that they possess free aldehyde (−CHO) or ketone (−C=O) groups that reduce weak oxidizing agents such as the copper in Benedict's reagent. **Benedict's reagent** contains cupric (copper) ion complexed with citrate in alkaline solution. Benedict's test identifies reducing sugars based on their ability to reduce the cupric (Cu^{2+}) ions to cuprous (Cu^{+}) oxide at basic (high) pH. Cuprous oxide is green to reddish orange.

Oxidized Benedict's reagent (Cu^{2+}) + Reducing sugar (R-COH)
(blue)

Heat
High pH ↓

Reduced Benedict's reagent (Cu^{+}) + Oxidized sugar (R-COOH)
(green to reddish orange)

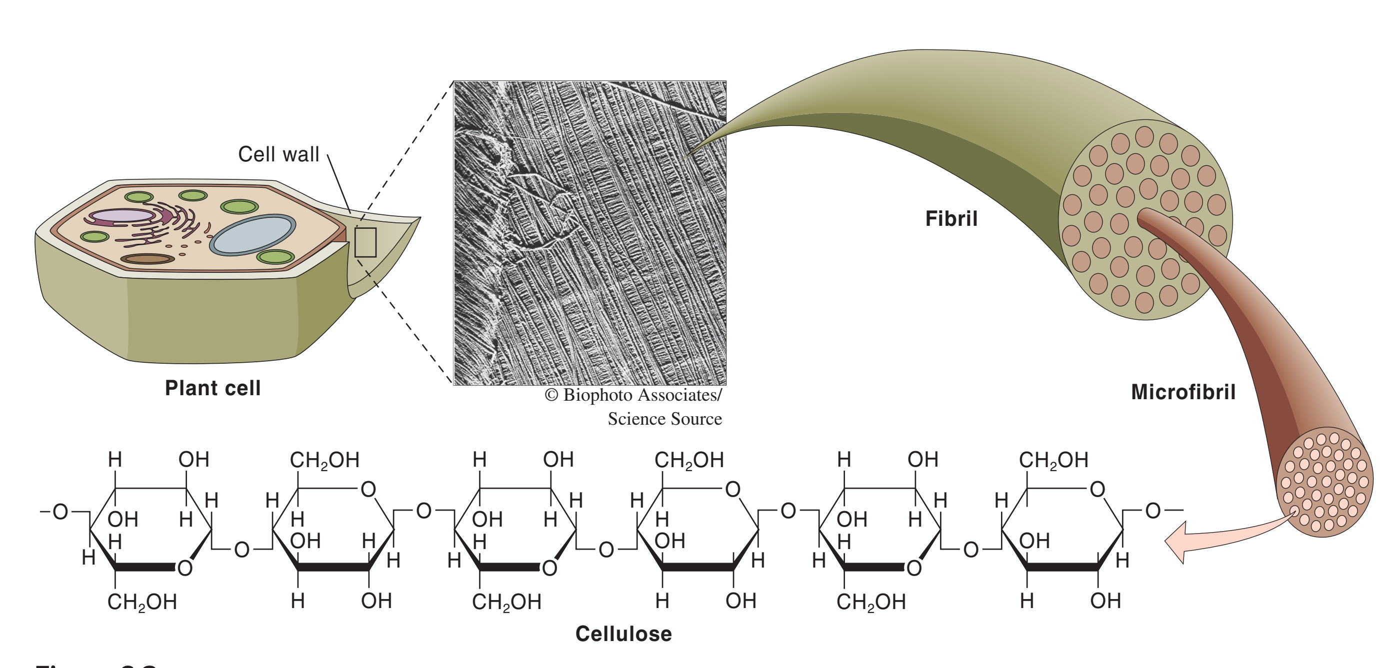


Figure 6.3 Plant cell walls made of cellulose arranged in fibrils and microfibrils. The scanning electron micrograph shows the fibrils in a cell wall of the green alga *Chaetomorpha* (30,000×).

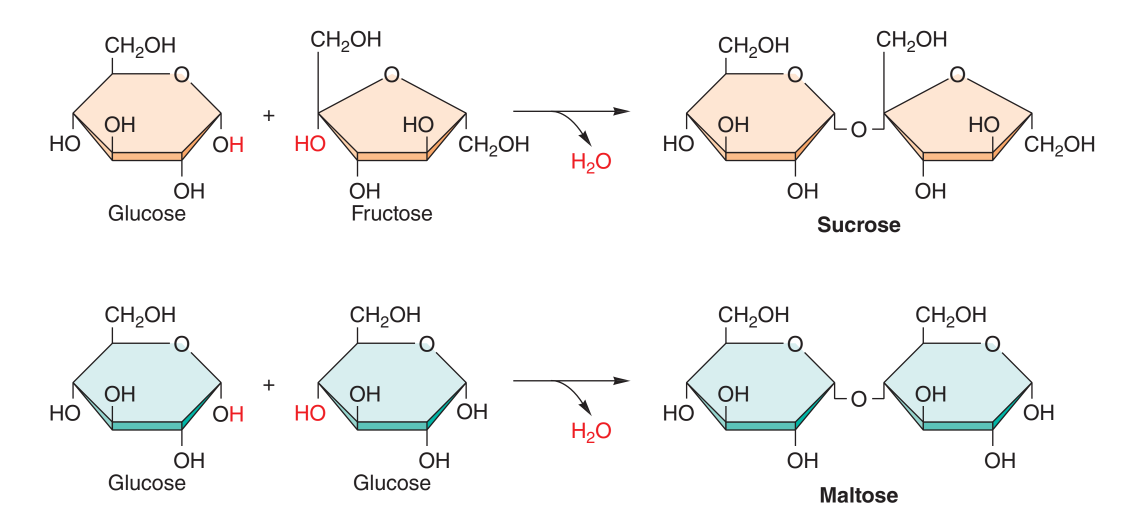


Figure 6.4 Dehydration synthesis is used to link monosaccharides (such as glucose and fructose) into disaccharides. The disaccharides shown here are maltose (malt sugar) and sucrose (table sugar).

A green solution indicates a small amount of reducing sugars, and reddish orange indicates an abundance of reducing sugars. Nonreducing sugars such as sucrose produce no change in color (i.e., the solution remains blue).



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 6.1 **Perform the Benedict's test for reducing sugars**

1. Obtain seven test tubes and number them 1–7.
2. Add to each tube the materials to be tested (the quantities of these materials are listed in table 6.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes. Add 2 mL of Benedict's solution to each tube.
3. Place all of the tubes in a gently boiling water-bath for 3 min and observe color changes during this time.
4. After 3 min, use a test-tube holder to remove the tubes from the water-bath. After giving the tubes ample time to cool to room temperature, record the color of their contents in table 6.1.
5. When you are finished, dispose of the contents of each tube as instructed by your instructor.

Question 2

a. Which of the solutions is a positive control? Negative control?

b. Which is a reducing sugar, sucrose or glucose? How do you know?

c. Which contains more reducing sugars, potato juice or onion juice? How do you know?

d. What does this tell you about how sugars are stored in onions and potatoes?

Iodine Test for Starch

Staining by iodine (iodine-potassium iodide, I_2KI) distinguishes starch from monosaccharides, disaccharides, and other polysaccharides. The basis for this test is that starch is a coiled polymer of glucose; iodine interacts with these coiled molecules and becomes bluish black. Iodine does not react with carbohydrates that are not coiled and remains yellowish

TABLE 6.1

SOLUTIONS AND COLOR REACTIONS FOR (1) BENEDICT'S TEST FOR REDUCING SUGARS AND (2) IODINE TEST FOR STARCH

TUBE	SOLUTION	BENEDICT'S COLOR REACTION	IODINE COLOR REACTION
1	10 drops onion juice		
2	10 drops potato juice		
3	10 drops sucrose solution		
4	10 drops glucose solution		
5	10 drops distilled water		
6	10 drops reducing-sugar solution		
7	10 drops starch solution		
8			
9			

brown. Therefore, a bluish-black color is a positive test for starch, and a yellowish-brown color (i.e., no color change) is a negative test for starch. Glycogen, a common polysaccharide in animals, has a slightly different structure than does starch and produces only an intermediate color reaction.

Procedure 6.2 **Perform the iodine test for starch**

1. Obtain seven test tubes and number them 1–7.
2. Add to each tube the materials to be tested (table 6.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Add three to six drops of iodine to each tube.
4. Record the color of the tubes' contents in table 6.1.

Question 3

a. Which of the solutions is a positive control? Which is a negative control?

b. Which colors more intensely, onion juice or potato juice? Why?

c. In what parts of a plant is the most starch typically stored?

d. What are the functions of carbohydrates in living organisms?

PROTEINS

Proteins are remarkably versatile structural molecules found in all life forms (fig. 6.5). Proteins are made of amino acids (fig. 6.6), each of which has an amino group (—NH_2), a carboxyl (acid) group (—COOH), and a variable side chain (—R). A **peptide bond** (fig. 6.7) forms between the amino group of one amino acid and the carboxyl group of an adjacent amino acid and is identified by a **Biuret test.** Specifically, peptide bonds (C—N bonds) in proteins complex with Cu^{2+} in Biuret reagent and produce a violet color. A Cu^{2+} must complex with four to six peptide bonds to produce a color; therefore, individual amino acids do not react positively. Long-chain polypeptides (proteins) have many peptide bonds and produce a positive reaction.

Biuret reagent is a 1% solution of $CuSO_4$ (copper sulfate). A violet color is a positive test for the presence of protein; the intensity of color relates to the number of peptide bonds that react.

Question 4

Examine figure 6.6. Shade with a pencil the reactive amino and carboxyl groups on the three common amino acids shown.

(a) Fibrin © Steve Gsvhmeissner/Science Photo Library/Getty Images

(b) Collagen © Craig Veltri/Getty Images

(c) Keratin © Jim Wehtje/Brand X Pictures/Punchstock

(d) Spider silk © Index Stock/Alamy

(e) Hair © Armina-Udovenko/Getty Images

Figure 6.5 Common structural proteins. (*a*) Fibrin. This electron micrograph shows a red blood cell caught in threads of fibrin (800×). Fibrin is important in the formation of blood clots. (*b*) Collagen. The so-called "cat-gut" strings of a tennis racket are made of collagen. (*c*) Keratin. This type of protein makes up bird feathers, such as this peacock feather. (*d*) Spider silk. The web spun by this agile spider is made of protein. (*e*) Hair. Hair is also a protein.

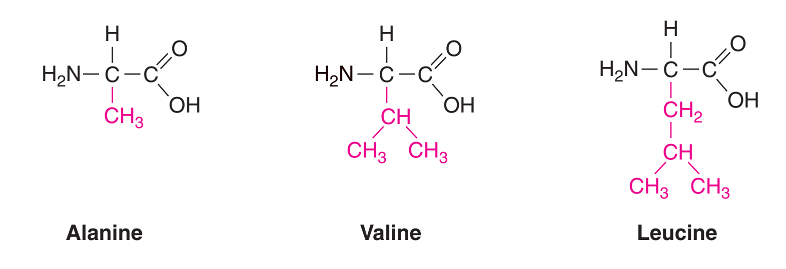


Figure 6.6 Structures of three amino acids common in proteins. Each amino acid has one carbon bonded to both an amine group (—NH_2) and a carboxyl group (—COOH). The side chains that make each amino acid unique are shown in red.

Procedure 6.3 Perform the Biuret test for protein

1. Obtain five test tubes and number them 1–5. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
2. Add the materials listed in table 6.2.
3. Add 2 mL of 2.5% sodium hydroxide (NaOH) to each tube.

TABLE 6.2

SOLUTIONS AND COLOR REACTIONS FOR THE BIURET TEST FOR PROTEINS

TUBE	SOLUTION	COLOR
1	2 mL egg albumen	
2	2 mL honey	
3	2 mL amino acid solution	
4	2 mL distilled water	
5	2 mL protein solution	
6		
7		

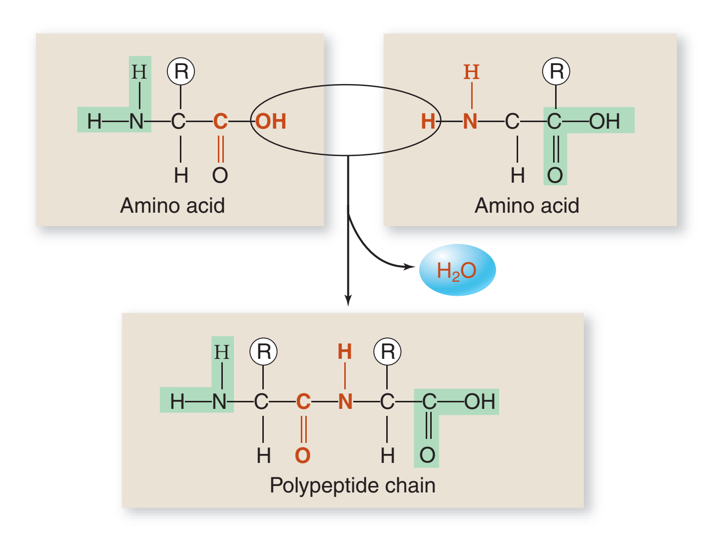


Figure 6.7 A peptide bond joins two amino acids, and peptide bonds link many amino acids to form polypeptides, or proteins. The formation of a peptide bond (i.e., between the carbon of one amino acid's carboxyl group and the nitrogen of another amino acid's amino group) liberates a water molecule. The R in these amino acids represents a variable side chain that characterizes each type of amino acid.



Do not spill the NaOH—it is extremely caustic. Rinse your skin if it comes in contact with NaOH.

4. Add three drops of Biuret reagent to each tube and mix.
5. Record the color of the tubes' contents in table 6.2.

Question 5

a. Which of the solutions is a positive control? Which is a negative control?

b. Which contains more protein (C—N bonds), egg albumen or honey? How can you tell?

c. Do free amino acids have peptide bonds?

d. What are the functions of proteins in living organisms?

LIPIDS

Lipids include a variety of molecules that dissolve in nonpolar solvents such as ether, acetone, methanol, or ethanol, but not as well in polar solvents such as water. Triglycerides (fats) are abundant lipids made of glycerol and three fatty acids (fig. 6.8). Tests for lipids are based on a lipid's ability to selectively absorb pigments in fat-soluble dyes such as Sudan IV.

Question 6

Examine figure 6.8. What are the reactive groups of the fatty acids?

Figure 6.8 The structure of a fat includes glycerol and fatty acids. (*a*) An ester linkage forms when the carboxyl group of a fatty acid links to the hydroxyl group of glycerol, with the removal of a water molecule. (*b*) Triacylglycerides are fats whose fatty acids vary in length and vary in the presence and location of carbon–carbon double bonds.



Handle acetone carefully; it is toxic.

Procedure 6.4 Solubility of lipids in polar and nonpolar solvents

1. Obtain two test tubes. To one of the tubes, add 5 mL of water. To the other tube, add 5 mL of acetone.
2. Add a few drops of vegetable oil to each tube.

Question 7

What do you conclude about the solubility of lipids in polar solvents such as water? In nonpolar solvents such as acetone?

Procedure 6.5 Perform the Sudan IV test for lipid

1. Obtain five test tubes and number them 1–5. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
2. Add the materials listed in table 6.3.
3. Add five drops of water to tube 1 and five drops of Sudan IV to each of the remaining tubes. Mix the contents of each tube. Record the color of the tubes' contents in table 6.3.

Question 8

a. Is salad oil soluble in water?

TABLE 6.3

SOLUTIONS AND COLOR REACTIONS FOR THE SUDAN IV TEST FOR LIPIDS

TUBE	SOLUTION	DESCRIPTION OF REACTION
1	1 mL salad oil + water	
2	1 mL salad oil + Sudan IV	
3	1 mL honey + Sudan IV	
4	1 mL distilled water + Sudan IV	
5	1 mL known lipid solution + Sudan IV	
6		
7		

b. Compare tubes 1 and 2. What is the distribution of the dye with respect to the separated water and oil?

c. What observation indicates a positive test for lipid?

d. Does honey contain much lipid?

e. Lipids supply more than twice as many calories per gram as do carbohydrates. Based on your results, which contains more calories, oil or honey?

Grease-Spot Test for Lipids

A simpler test for lipids is based on their ability to produce translucent grease marks on unglazed paper.

Procedure 6.6 **Perform the grease-spot test for lipids**

1. Obtain a piece of brown wrapping paper or brown paper bag from your lab instructor.
2. Use an eyedropper to add a drop of salad oil near a corner of the piece of paper.
3. Add a drop of water near the opposite corner of the paper.
4. Let the fluids evaporate.
5. Look at the paper as you hold it up to a light.
6. Test other food products and solutions available in the lab in a similar way and record your results in table 6.4.

TABLE 6.4

MATERIALS AND GREASE-SPOT REACTION AS A TEST FOR LIPID CONTENT

FOOD PRODUCT	DESCRIPTION OF GREASE-SPOT REACTION
1	
2	
3	
4	
5	
6	

TABLE 6.5

SOLUTIONS AND COLOR REACTIONS FOR DISCHE DIPHENYLAMINE TEST FOR DNA

TUBE	SOLUTION	COLOR
1	2 mL DNA solution	
2	1 mL DNA solution, 1 mL water	
3	2 mL RNA solution	
4	2 mL distilled water	
5		
6		

Question 9

a. Which of the food products that you tested contain large amounts of lipid?

b. What are the functions of lipids in living organisms?

NUCLEIC ACIDS

DNA and RNA are nucleic acids made of nucleotide subunits (fig. 6.9). DNA can be identified chemically with the **Dische diphenylamine test.** Acidic conditions convert deoxyribose to a molecule that binds with diphenylamine to form a blue complex. The intensity of the blue color is proportional to the concentration of DNA.

Procedure 6.7 **Perform the Dische diphenylamine test for DNA**

1. Obtain four test tubes and number them 1–4. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.

2. Add the materials listed in table 6.5.

3. Add 2 mL of the Dische diphenylamine reagent to each tube and mix thoroughly.



Handle the Dische diphenylamine reagent carefully; it is toxic. Wash your hands after the procedure.

4. Place the tubes in a gently boiling water-bath to speed the reaction.

5. After 10 min, transfer the tubes to an ice bath. Gently mix and observe the color of their contents as the tubes cool. Record your observations in table 6.5.

Question 10

a. How does the color compare between tubes 1 and 2? Why?

b. Do DNA and RNA react alike? Why or why not?

c. What are the functions of nucleic acids in living organisms?

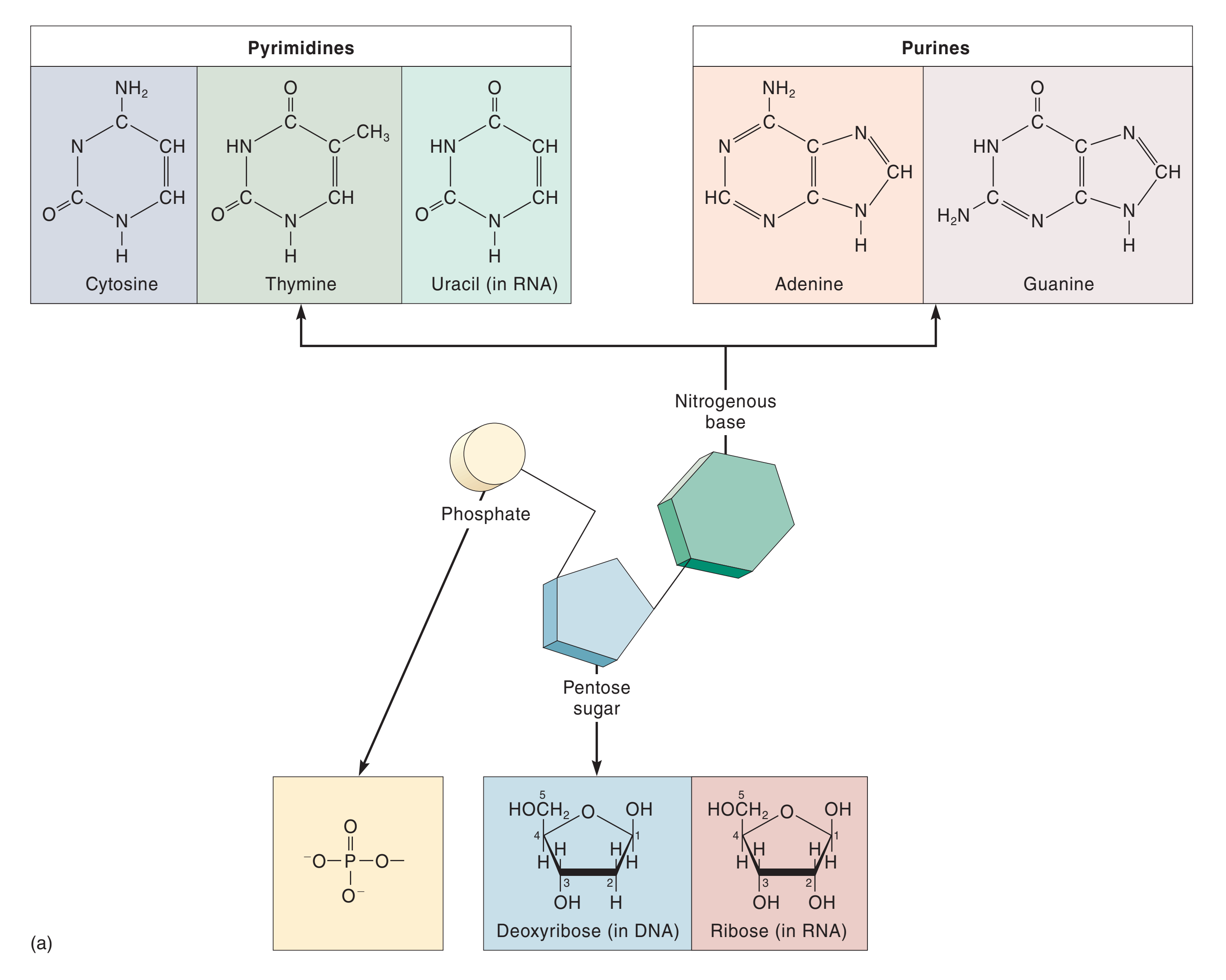


Figure 6.9 The structure of DNA and RNA. (*a*) Each nucleotide consists of three smaller building blocks: a nitrogenous base, a pentose sugar, and a phosphate group. (*b*) Nucleotides are bonded to each other by covalent bonds between the phosphate of one nucleotide and the sugar of the next nucleotide. (*c*) DNA is usually a double strand held together by hydrogen bonds between nitrogenous bases; A bonds only with T, and C bonds only with G. The double strand is twisted into a double helix.

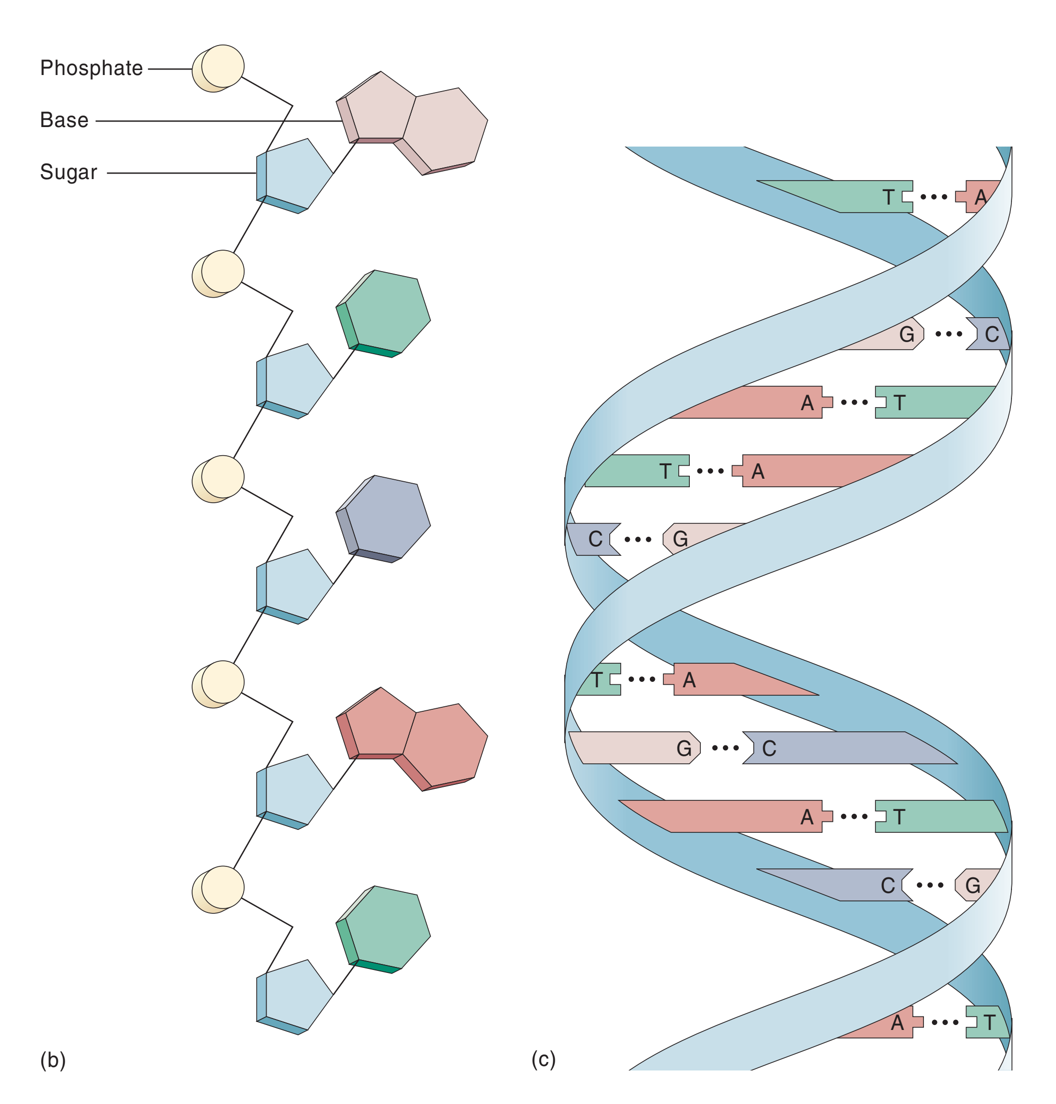


Figure 6.9 continued

INVESTIGATION I

Identify Unknowns

Each of the previously described tests is relatively specific; that is, iodine produces a bluish-black color with starch but not with other carbohydrates, protein, lipid, or nucleic acids. This specificity can be used to identify the contents of an unknown solution.

a. Obtain an unknown solution from your laboratory instructor. Record its number in table 6.6.
b. Obtain 10 clean test tubes.
c. Number five tubes for the sample as S1–S5. Number the other five tubes as controls C1–C5.
d. Place 2 mL of your unknown solution into each of tubes S1–S5.
e. Place 2 mL of distilled water into each of tubes C1–C5.
f. Use procedures 6.1–6.5 to detect reducing sugars, starch, protein, DNA, and lipids in your unknown. Your unknown may contain one, none, or several of these macromolecules. Record your results in table 6.6. Show table 6.6 and the following report (page 69) to your instructor before you leave the lab.

INVESTIGATION II

Variation in Starch Storage by Roots versus Leaves

Observation: Starch is the major storage product of photosynthesis in higher plants, and some plant organs more than others are specialized for storing starch. Iodine reacts with starch to produce a dark blue-black color.

Question: What are the relative amounts of starch stored in leaves versus roots of a flowering plant?

a. Establish a working lab group and obtain Investigation Worksheet 6 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 6 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Name ____________________ Lab Section ____________

Unknown No. ____________

Table 6.6

Chemical Testing to Identify an Unknown

Biochemical Test	Color		Unknown Result
	Sample	Control	(+/−)
Benedict's test (reducing sugars)			
Iodine (starch)			
Biuret test (protein)			
Dische diphenylamine test (DNA)			
Sudan IV (lipid)			

Report: Identity of Unknown

Indicate which of the following are in your unknown:

Reducing sugars

Starch

Protein

DNA

Lipid

Comments:

Questions for Further Thought and Study

1. What is the importance of a positive control? What is the importance of a negative control?

2. What controls were used in each procedure that you performed in today's lab?

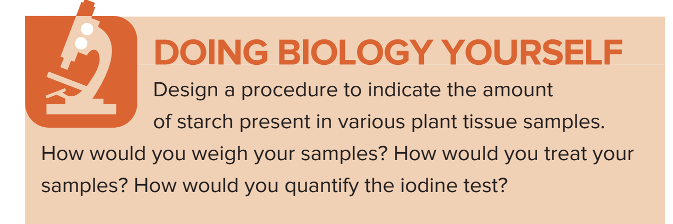
3. Why did you include controls in all of your tests?

4. Are controls always necessary? Why or why not?

5. What is a phospholipid? What functions do phospholipids have in cells?

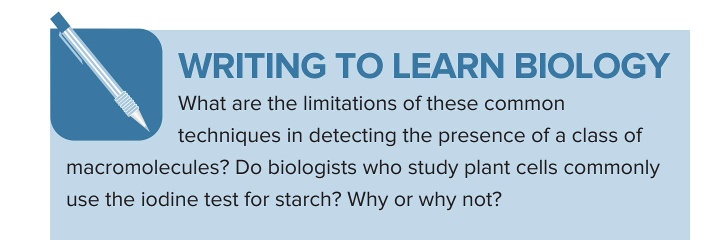
6. What does a "dehydration synthesis" do?

7. Food labels list the amounts of (and calories from) carbohydrates, fats, and proteins, but not nucleic acids. Why not?



DOING BIOLOGY YOURSELF

Design a procedure to indicate the amount of starch present in various plant tissue samples. How would you weigh your samples? How would you treat your samples? How would you quantify the iodine test?



WRITING TO LEARN BIOLOGY

What are the limitations of these common techniques in detecting the presence of a class of macromolecules? Do biologists who study plant cells commonly use the iodine test for starch? Why or why not?

Exercise 7

Separating Organic Compounds

Column Chromatography, Paper Chromatography, and Gel Electrophoresis

Learning Objectives

By the end of this exercise you should be able to:

1. Describe the basis for column chromatography, paper chromatography, and gel electrophoresis.
2. Use column chromatography, paper chromatography, and gel electrophoresis to separate organic compounds from mixtures.



Please visit connect.mheducation.com to review online resources tailored to this lab.

Cells are a mixture of the types of organic compounds that you studied in Exercise 6 ("Biologically Important Molecules"), including carbohydrates, proteins, lipids, and nucleic acids. Biologists characterize and study these compounds to understand how organisms function. This requires that biologists separate the compounds, such as amino acids and nucleotides, from mixtures. Three separation techniques that biologists use are column chromatography, paper chromatography, and gel electrophoresis.

In today's exercise you will use these common techniques to separate compounds from mixtures. The procedures are simple and model how these techniques are used by biologists in their research.

COLUMN CHROMATOGRAPHY

Column chromatography separates molecules according to their size and shape. The procedure is simple and involves placing a sample onto a column of beads having tiny pores. Molecules can move through the column of beads in two ways: a fast route between the beads or a slower route through the tiny pores of the beads. Molecules too big to fit into the beads' pores move through the column quickly, whereas smaller molecules enter the beads' pores and move through the column more slowly (fig. 7.1). Movement of the molecules is analogous to going through or walking around a maze: It takes more time to walk through a maze than to walk around it.

The apparatus used for column chromatography is shown in figure 7.2 and consists of a chromatography column, a matrix, and a buffer.

- The **chromatography column** is a tube having a frit and a spout at its bottom. The frit is a membrane or porous disk that supports and keeps the matrix in the column but allows water and solutes to pass.
- The **matrix** is the material in the column that fractionates, or separates, the chemicals mixed in the sample. The matrix consists of beads having tiny pores and internal channels. The size of the beads' pores determines the matrix's **fractionation range,** which is the range of molecular weights the matrix can separate. These molecular weights are measured in units called daltons; 1 dalton $\approx$ 1 g $mole^{-1}$. Different kinds of matrices have different fractionation ranges. In today's exercise you'll use a matrix having a fractionation range of 1000 to 5000 daltons. As they move through the matrix, small molecules spend much time in the maze of channels and pores in the matrix. Large molecules do not.
- The **buffer** helps control the pH of the sample (see Exercise 5). A buffer is a solution with a known pH that resists changes in pH if other chemicals are added. The pH of a buffer remains relatively constant. This is important because the shapes of molecules such as proteins often vary according to their pH. The buffer carries the sample through the matrix, which separates the chemicals mixed in the sample.

Column chromatography can also separate compounds having the same molecular weight but different shapes. Compact, spherical molecules penetrate the pores and channels of the matrix more readily than do rod-shaped molecules. Thus, spherical molecules move through a column more slowly than do rod-shaped molecules.

During column chromatography, the buffer containing the sample mixture of chemicals moves through the column and is collected sequentially in test tubes from the bottom of the column. Biologists then assay the content of the tubes to determine which tubes contain the compounds in which they are interested.

Chemical mixture is added to column.

Beads exclude large molecules.

Porous beads

Organic molecules separate by size; larger molecules move fastest and therefore appear in the earlier fractions.

1 2 3 4 5 6

Figure 7.1 Separation of organic molecules by column chromatography. As the solution flows through the column, the smaller molecules are slowed down as they pass through the pores of the beads. Medium-sized molecules will pass through a bead with pores less frequently, and the largest molecules will quickly flow around all the beads. The exiting fluid is collected in fractions. The first fractions collected will contain the largest molecules.



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Question 1

In today's exercise you'll isolate colored compounds from mixtures. However, most biological samples are colorless. How would you determine the contents of the test tubes if all of the samples were transparent?

Procedure 7.1 Separate compounds by column chromatography

1. Label nine microtubes 1–9.
2. Obtain an apparatus for column chromatography and carefully remove all of the buffer from above the beads with a transfer pipet. Do not remove any of the matrix.
3. Obtain a sample to be separated. The sample is a mixture of Orange G (molecular weight = 452 g mole^{-1}) and a rodlike polymer of glucose stained blue and having a molecular weight of about 2,000,000 g mole^{-1}.
4. Use a transfer pipet to slowly load 0.2 mL of the sample onto the top of the beads. Drip the sample down the inside walls of the column.
5. Place a beaker under the column.
6. Slowly open the valve. This will cause the sample to enter the beads. Close the valve after the sample has



Figure 7.2 Apparatus for column chromatography. A fraction is being collected, drop by drop, in the beaker.

completely entered the beads (i.e., when the top of the beads is exposed to air).

7. Use a transfer pipet to slowly cover the beads with buffer. Add buffer until the reservoir is almost full.

8. Hold microtube 1 under the column and open the valve until you've collected about 1.0 mL of liquid.

9. Repeat step 8 for tubes 2–9. The sample will separate in the column.

10. Identify the tubes containing (1) the most orange dye, and (2) the most blue dye that eluted from the column.

11. Refill the reservoir with buffer and cover the reservoir with Parafilm.

Question 2

a. Was the color separation distinctive? Would you expect a longer column to more clearly separate the compounds? Why or why not?

b. Suppose your sample had consisted of a mixture of compounds having molecular weights of 50,000, 100,000, and 1,000,000 g $mole^{-1}$. What type of results would you predict? Explain your answer.

PAPER CHROMATOGRAPHY

Biologists often analyze the amino acid content of samples to determine protein sequences and enzyme structures. Amino acids can be separated by partitioning them between the stationary and mobile phases of paper chromatography. The **stationary phase** is the paper fibers, and the **mobile phase** is an organic solvent that moves along the paper.

Separation by paper chromatography begins by applying a liquid sample to a small spot on an origin line at one end of a piece of chromatography paper. The edge of the paper is then placed in a solvent. As the solvent moves up the paper, any sample molecules that are soluble in the solvent will move with the solvent. However, some molecules move faster than others based on their solubility in the mobile phase and their attraction to the stationary phase. These competing factors are different for different molecular structures, so each type of molecule moves at a different speed and occurs at a different position on the finished chromatogram.

Amino acids in solution have no color but react readily with molecules of ninhydrin to form a colored product. A completed chromatogram is sprayed with a ninhydrin solution and heated to detect the amino acids. The distance of these spots from the origin is measured and used to quantify the movement of a sample. The resulting R_f value (retardation factor) characterizes a known molecule in a known solvent under known conditions, and is calculated as follows:

$$R_f = \frac{\textbf{Distance moved by sample}}{\textbf{Distance from origin to solvent front}}$$

Procedure 7.2 **Separate amino acids and identify unknowns by paper chromatography**

1. Obtain a piece of chromatography paper 15 cm square. Avoid touching the paper with your fingers. Use gloves, tissue, or some other means to handle the paper because oils from your skin will alter the migration of the molecules on the paper.

2. Lay the paper on a clean paper towel. Then use a pencil to draw a light line 2 cm from the bottom edge of the paper.

3. Draw five tick marks at 2.5 cm intervals from the left end of the line. Lightly label the marks 1–5 below the line.

Table 7.1

Chromatography Data for Determining Amino Acid Unknowns

Tick Mark Number	Amino Acid or Sample Number	Distance to Solvent Front	Distance Traveled by Sample	R_f	Identity of Unknown
1					
2					
3					
4					
5					

4. Locate the five solutions available for the chromatography procedure. Three of the solutions are known amino acids. One solution is an unknown. The last solution is a plant extract or another unknown.
5. Use a wooden or glass applicator stick to "spot" one of the solutions on mark #1. To do this, dip the stick in the solution and touch it to the paper to apply a small drop (2–3 mm in diameter). Let the spot dry; then make three to five more applications on the same spot. Dry between each application. Record in table 7.1 the name of the solution next to the appropriate mark number.
6. Repeat step 5 for each of the other solutions.
7. Staple or paper clip the edges of the paper to form a cylinder with the spots on the outside and at the bottom.
8. Obtain a quart jar containing the chromatography solvent. The solvent should be 1 cm or less deep. The solvent consists of butanol, acetic acid, and water (2:1:1).
9. Place the cylinder upright in the jar (fig. 7.3). *The solvent must be below the pencil line and marks*. Close the lid to seal the jar.
10. Keep the jar out of direct light and heat. Allow the solvent to move up the paper for 2 hours (h) but not all the way to the top.
11. Open the jar and remove the chromatogram. Unclip and flatten the paper. Dry it with a fan or hair dryer. Work under a fume hood if possible to avoid breathing the solvent vapors.
12. Spray the chromatogram with ninhydrin. Carefully dry the chromatogram with warm air.
13. Circle with a pencil each of the spots. Measure the distance each of the spots has traveled and calculate the R_f for each spot. Record the values in table 7.1.
14. Determine the contents of the unknown solutions by comparing R_f values. Record the results in table 7.1.

GEL ELECTROPHORESIS

Gel electrophoresis separates molecules according to their charge, shape, and size (fig. 7.4). Buffered samples (mixtures of organic chemicals) are loaded into a Jello-like gel, after which an electrical current is placed across the gel. This current moves the charged molecules toward either the cathode or anode of the electrophoresis apparatus. The speed, direction, and distance that each molecule moves are related to its charge, shape, and size.

The apparatus for gel electrophoresis is shown in figure 7.5 and consists of an electrophoresis chamber, gel, buffer, samples, and a power supply.

- The gel is made by dissolving agarose powder (a derivative of agar) in hot buffer. When the solution cools, it solidifies into a gel having many pores that function as a molecular sieve. The gel is submerged in a buffer-filled chamber containing electrodes.
- The buffer conducts electricity and helps control the pH. The pH affects the stability and charge of the samples.
- The samples are mixtures of chemicals loaded into wells in the gel. These samples move in the gel during electrophoresis. Samples are often mixed with glycerol

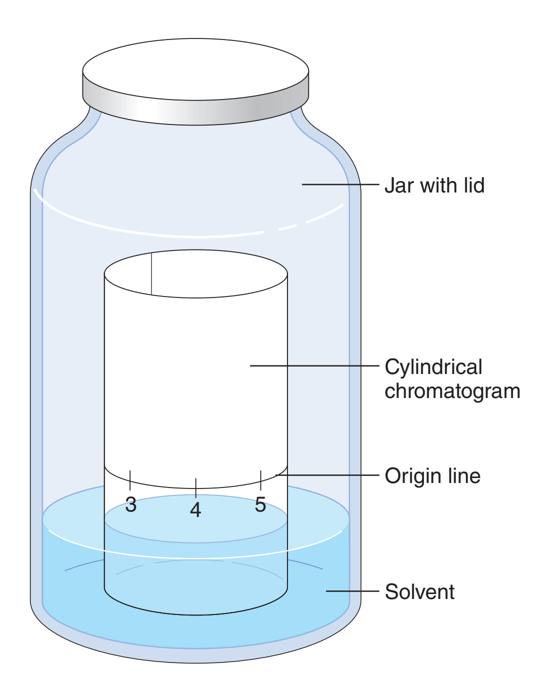


Figure 7.3 Apparatus for paper chromatography. Numbers on the chromatogram indicate the positions of multiple samples applied to the chromatogram. The samples will move up the chromatogram along with the solvent.

or sucrose to make them denser than the buffer so that they will not mix with the buffer.

- The power supply provides a direct current across the gel. Charged molecules respond to the current by moving from the sample wells into the gel. Negatively charged molecules move through the gel toward the positive electrode (anode), whereas positively charged molecules move through the gel toward the negative electrode (cathode). The greater the voltage, the faster the molecules move.

The sieve properties of the gel affect the rate of movement of a sample through the gel. Small molecules move more easily through the pores than do larger molecules. Consequently, small, compact (e.g., spherical) molecules move faster than do large, rodlike molecules. If molecules have similar shapes and molecular weights, the particles having the greatest charge move fastest and, therefore, the farthest.

Procedure 7.3 Separate organic molecules by gel electrophoresis

1. Obtain an electrophoresis chamber. Cover the ends of the bed as shown in figure 7.6 and demonstrated by your instructor.
2. Place a six-tooth comb in or near the middle set of notches of the gel-cast bed. There should be a small space between the bottom of the teeth and the bed.

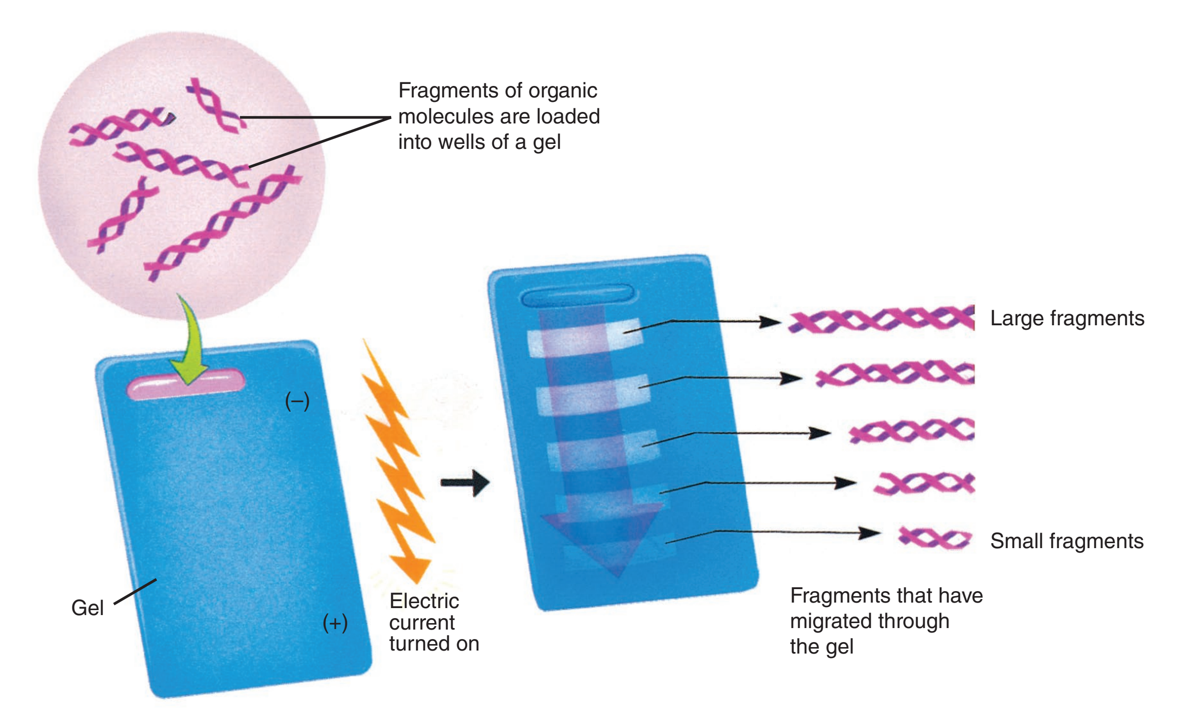
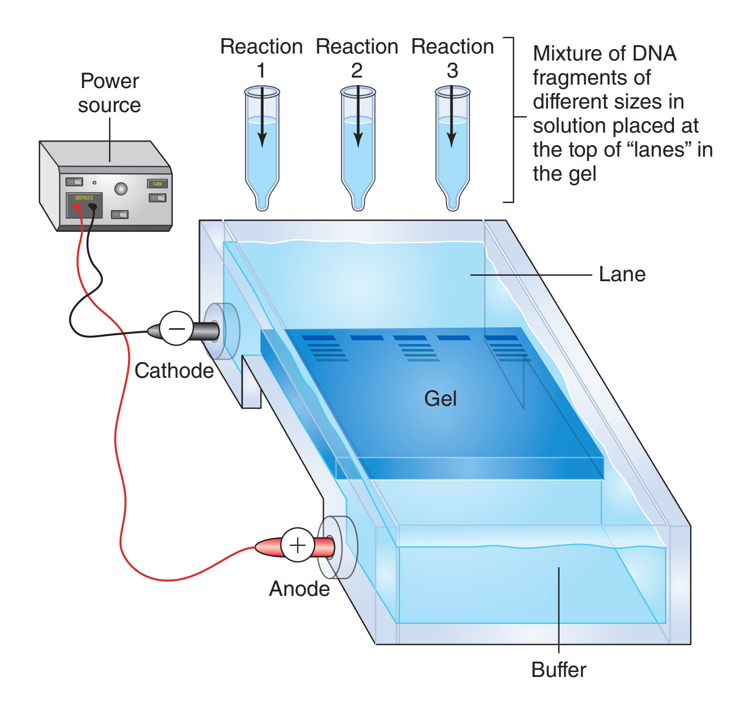


Figure 7.4 Gel electrophoresis. This process separates DNA fragments, protein fragments, and other organic compounds by causing them to move through an electrically charged gel. The fragments move according to their size, shape, and electrical charge; some fragments move slowly and some move quickly. When their migration is complete, the fragments can be stained and visualized easily. In the example shown here, the DNA fragments were separated by size.

Figure 7.5 Apparatus for gel electrophoresis. The power supply produces an electrical gradient between the + and − poles and across the gel.





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Figure 7.6 Cover the ends of the removable gel bed with rubber end-caps or tape.

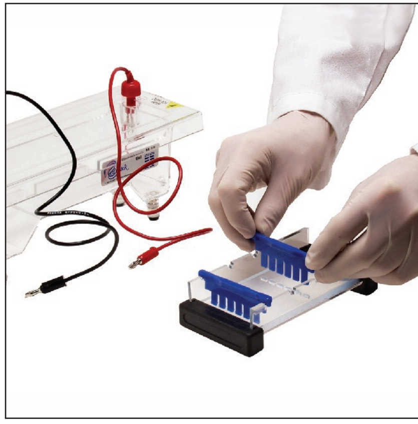


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Figure 7.7 Place comb near the center set of notches of the gel bed. Prepare the agarose solution and pour the gel.

3. Mix a 0.8% (weight by volume) mixture of agarose powder in a sufficient volume of buffer to fill the gel chamber. Heat the mixture until the agarose dissolves.
4. When the hot agarose solution has cooled to 50°C, pour the agarose solution into the gel-cast bed (fig. 7.7).
5. After the gel has solidified, gently remove the comb by pulling it straight up (fig. 7.8). Use of a plastic spatula may help prevent tearing the gel. Use the sketch in figure 7.9 to label the wells formed in the gel by the comb.
6. Submerge the gel under the buffer in the electrophoresis chamber.
7. You will study six samples:

   Sample 1: Bromophenol blue (molecular weight = 670 g mole^{-1})



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Figure 7.8 After the gel solidifies, gently remove the rubber end-caps (or tape) and pull the combs straight up from the gel.

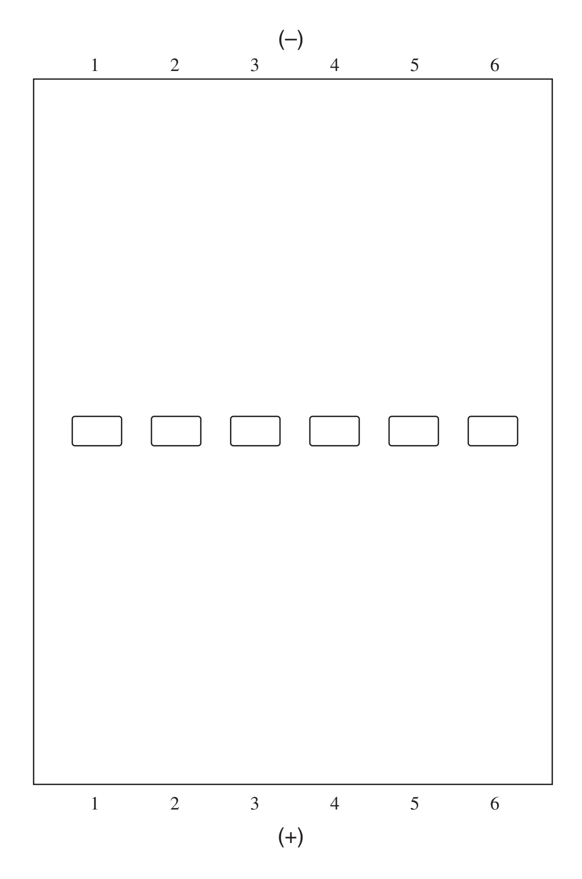


Figure 7.9 Sketch of the wells formed in the gel by the comb as viewed from above.

Sample 2: Methylene blue (molecular weight = 320 g $mole^{-1}$)

Sample 3: Orange G (molecular weight = 452 g $mole^{-1}$)

Sample 4: Xylene cyanol (molecular weight = 555 g $mole^{-1}$)

Samples 5 and 6: Unknowns

Use a micropipettor or a simple pipet and bulb to load the samples into the wells of the gel. If you use a micropipettor, your instructor will demonstrate its use. If you use a simple pipet and bulb, gently squeeze the pipet bulb to draw Sample 1 into the pipet. Be sure that the sample is in the lower part of the pipet. If the sample becomes lodged in the bulb, tap the pipet until the sample moves into the lower part.

8. To eliminate excess air, hold the pipet above the sample tube and slowly squeeze the bulb until the sample is near the pipet's opening.
9. Place the pipet tip into the electrophoresis buffer so it is barely inside sample well 1 (fig. 7.10). Do not touch the bottom of the sample well. Maintain pressure on the pipet bulb to avoid pulling buffer into the pipet.
10. Slowly inject the sample into the sample well. Stop squeezing the pipet when the well is full. Do not release the pressure on the bulb. Remove the pipet from the well.
11. Thoroughly rinse the pipet with distilled water.
12. Load the remaining five samples into the gel by repeating steps 6–10 (fig. 7.10). Load Sample 2 into the second well, Sample 3 into the third well, etc.
13. Carefully snap on the cover of the electrophoresis chamber (fig. 7.11). The red plug in the cover should be placed on the terminal indicated by the red dot. The black plug in the cover should be placed on the terminal indicated by the black dot.
14. Insert the plug of the black wire into the black (negative) input of the power supply. Insert the plug of the red wire into the red (positive) input of the power supply.
15. Turn on the power and set the voltage at 90 V. You'll soon see bubbles forming on the electrodes. Examine the gel every 10 min.
16. After 30 min, turn off the power and disconnect the leads from the power source. Gently remove the cover from the chamber and sketch your results in figure 7.9.

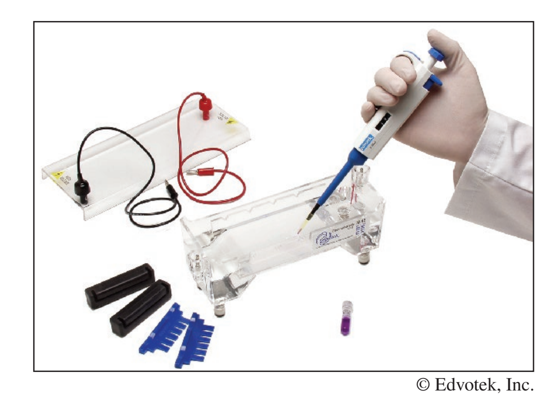


Figure 7.10 Submerge the gel in the buffer-filled electrophoresis chamber and load the samples into the wells of the gel.

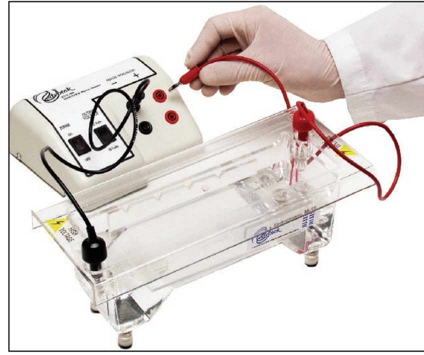


Figure 7.11 Attach the safety cover, connect the power source, and run the electrophoresis.

Question 3

a. Bromophenol blue, Orange G, and xylene cyanol each have a negative charge at neutral pH, whereas methylene blue has a positive charge at neutral pH. How does this information relate to your results?

b. Did Orange G, bromophenol blue, and xylene cyanol move the same distance in the gel? Why or why not?

c. What compounds do you suspect are in Samples 5 and 6? Explain your answer.

INTERPRETING A DNA-SEQUENCING GEL

Examine figure 7.12, which includes a photograph of a gel used to determine the order, or sequence, of nucleotides in a strand of DNA. To prepare the sample for electrophoresis,

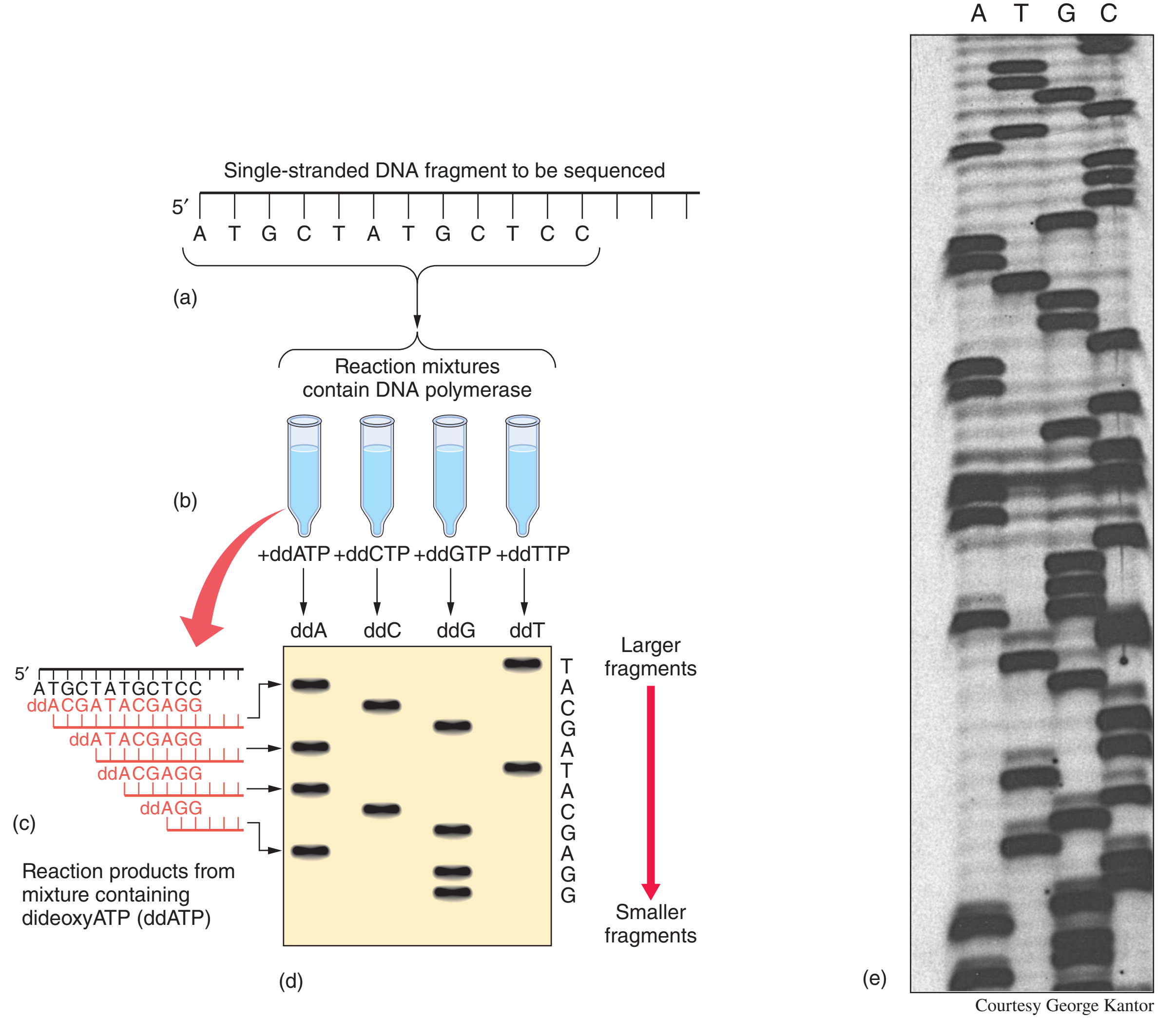


Figure 7.12 Determining the sequence of nucleotides in DNA. (*a*) Treating DNA with sodium hydroxide (NaOH) denatures double-stranded DNA into single-stranded DNA. One of the single strands of DNA to be sequenced is placed in each of four tubes. (*b*) The enzyme DNA polymerase is added to each tube along with a specific nucleotide-terminator. As polymerase replicates the DNA, the terminators are incorporated and will terminate various lengths of fragments of DNA. For example, the terminator ddATP will halt the reaction wherever adenosine occurs. The terminator ddATP (dideoxy adenosine triphosphate) will terminate a growing strand because it lacks a 3′ hydroxyl group and therefore cannot bond with the next deoxynucleotide. (*c*) Each tube will contain a sample of all possible replicated fragment lengths corresponding to the positions of that specific nucleotide. The sequences in red are the complement strands. (*d*) During electrophoresis, the fragments migrate at different rates according to their length. (*e*) The lanes of the resulting gel are labeled according to their base: A, adenine; T, thymine; G, guanine; and C, cytosine. This technique is usually referred to as "Sanger" sequencing in honor of Fred Sanger, a Nobel laureate who, in 1977, first sequenced a piece of DNA.

samples of the DNA being investigated were put into each of four tubes and induced to replicate. Also, into the first tube, an adenine-terminator was added in addition to all the other nucleotides. As the complementary strand was being constructed, the terminators were occasionally incorporated wherever an adenine nucleotide was used. This random incorporation resulted in all possible lengths of DNA pieces that had an adenine on the end. The same process was conducted in the other tubes with thymine-, guanine-, and cytosine-terminators; one treatment for each of the four lanes in the gel. Electrophoresis separated the replicated pieces of DNA by size. Staining the gel revealed which lengths of the complementary DNA were terminated by which nucleotide-terminators. Examine figure 7.12d.

The gel consists of four "lanes," labeled A, T, G, and C, indicating either adenine-, thymine-, guanine-, or cytosine-terminated pieces of DNA. By "reading" down the gel, you can determine the sequence of nucleotides in the DNA. For example, the uppermost band of the gel is in the T (thymine) lane. Therefore, the first base of the piece of DNA is thymine. Similarly, the next bands are in the A, C, G, and A lanes. Thus, the first five bases of the complementary strand DNA are T-A-C-G-A. List the next seven nucleotides of the DNA as indicated by the gel. Also list the sequence of the first 12 nucleotides in the original DNA being investigated.

Question 4

a. How did the sequence of nucleotides revealed on the gel differ from the sequence of the original strand of DNA?

b. Assume that the gel shown in Figure 7.12d is from blood collected at a murder scene. This blood does not match that of the victim. You have collected DNA from five people suspected of murder. Gels comparable to the one shown in Figure 7.12d read as follows for each of the suspects:

Suspect #1: T-A-C-G-A-T-A-C-G-A-C
Suspect #2: T-A-C-G-A-T-A-C-G-A-C
Suspect #3: T-A-C-G-A-C-A-C-G-C-G
Suspect #4: T-A-C-G-A-T-G-C-G-A-C
Suspect #5: T-A-C-G-A-T-C-C-G-T-C

What do you conclude from this evidence?

INVESTIGATION I

Refining the Paper Chromatography Procedure

Carefully planned and refined procedures are critical for laboratory techniques such as paper chromatography. The sensitivity of these techniques depends on a variety of factors, including the many parameters associated with timing, chemicals, measurements, and temperatures. In procedure 7.2 you were given a rather standardized protocol, but it can always be improved for specific experiments. For example, how would you modify the paper chromatography procedure to better resolve two amino acids having approximately the same R_f values? What parameter(s) of the experimental design might be tweaked to increase the technique's resolving power? We suggest that you begin your investigation in the following way:

a. List the parameters involved in paper chromatography. Think carefully; many factors are involved.

b. Choose one or two parameters that you can test for their impact on the chromatography results. Why did you choose these?

c. Choose two amino acids for experimentation. Why did you choose these two?

d. Choose your treatment levels for each parameter, and then do your experiment.

e. What did you conclude?

INVESTIGATION II

The Importance of the Length of the Column in Column Chromatography

Observation: Column chromatography is a common means of separating molecules according to their size and shape. The movement of molecules through a column is affected by several factors, including the column's matrix and the column's length.

Question: How does the length of a column affect the separation of molecules via column chromatography?

a. Establish a working lab group and obtain Investigation Worksheet 7 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 7 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. How are column chromatography, paper chromatography, and gel electrophoresis different? How are they similar?

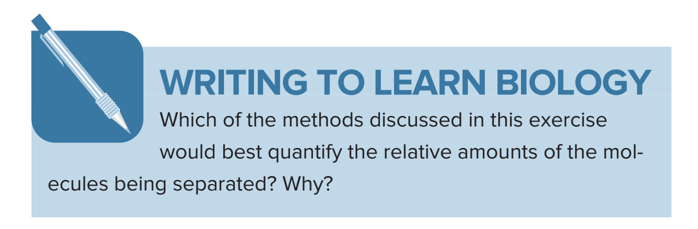
2. How would the results of electrophoresis vary if the voltage was increased? If the agarose was made more dense? Or if the migration was allowed to run twice as long?

3. How could knowing the nucleotide base sequence of a piece of DNA be important to a biologist?

4. How could knowing the nucleotide base sequence of a piece of DNA be important to someone trying to solve a crime?

5. How could knowing the nucleotide base sequence of a piece of DNA be important for someone studying a hereditary disease?

6. How could knowing the nucleotide base sequence of a piece of DNA be important for someone wanting to improve the yield of a crop such as corn?



WRITING TO LEARN BIOLOGY

Which of the methods discussed in this exercise would best quantify the relative amounts of the molecules being separated? Why?

Exercise 8

Spectrophotometry

Identifying Solutes and Determining Their Concentration

Learning Objectives

By the end of this exercise you should be able to:

1. Operate a spectrophotometer.
2. Describe the parts of a spectrophotometer and the function of each.
3. Construct absorption spectra for cobalt chloride and chlorophyll.
4. Construct and use a standard curve to determine the unknown concentration of a dissolved chemical.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Absorption and reflection of different wavelengths of light are part of your everyday experience. Different materials absorb and reflect different wavelengths of light; therefore they have different colors. When you recognize things with color you are observing that they absorb and reflect different wavelengths of light. A red object reflects red wavelengths to your eyes and absorbs the other wavelengths.

Light includes the visible wavelengths of the electromagnetic spectrum and is only a small part of the total spectrum. The entire electromagnetic spectrum includes radiation with wavelengths from less than 1 to more than 1 million nanometers. Visible light represents wavelengths between 380 and 700 nm (fig. 8.1). In this exercise you will work within the visible portion of the electromagnetic spectrum.

Question 1

a. Chlorophyll reflects green light (540–560 nm) and absorbs other wavelengths. What biologically important molecules other than chlorophyll absorb and reflect certain colors?

b. Is the absorbance of light critical to these molecules' functions or just a consequence of their molecular structure? Explain your answer.

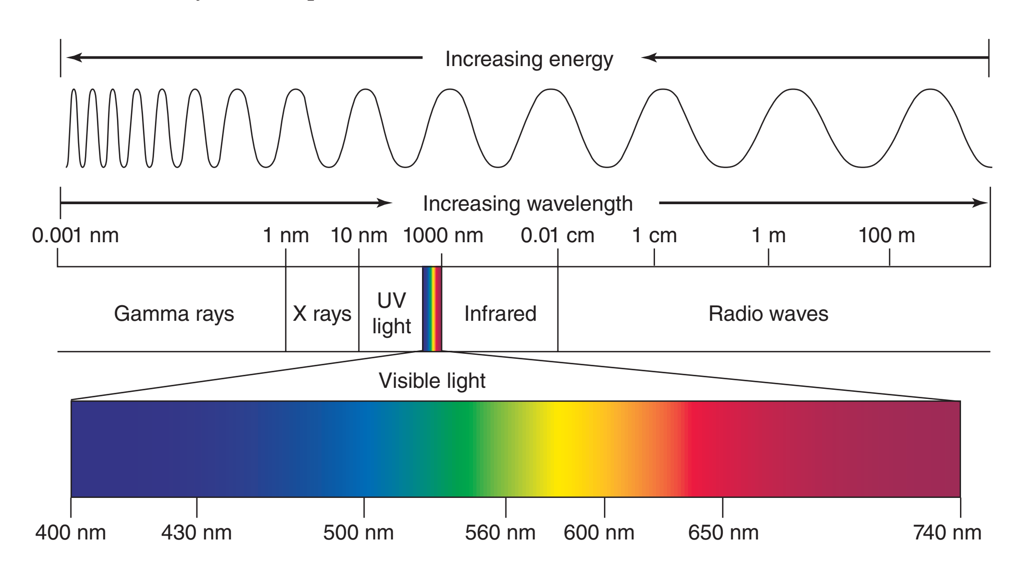


Figure 8.1 The electromagnetic spectrum. Light is a form of electromagnetic energy and is conveniently thought of as a wave. The shorter the wavelength of light, the greater the energy. Visible light, which represents a small part of the electromagnetic spectrum, occurs between 380 and 700 nanometers (nm). (UV stands for ultraviolet light.) Also see figure 8.5.

Spectrophotometry is one of our most versatile and precise techniques for assays ranging from blood chemistry to pollutants in lake water. Spectrophotometry is based on the principle that every different atom, molecule, or chemical bond absorbs unique wavelengths of light. For example, the nitrogenous base cytosine absorbs a different pattern of light than does adenine, uracil, or any other molecule with a different structure. As part of this pattern, some wavelengths are absorbed and some are not absorbed. Conversely, a unique pattern of light is reflected as well as absorbed by each chemical. Each chemical has a unique pattern or "fingerprint" of various wavelengths that it absorbs and/or reflects. In this exercise you will (a) determine the unique pattern of absorption for two common molecules, and then (b) use spectrophotometry to measure the concentrations of one of these molecules.

SPECTROPHOTOMETER

An instrument called a **spectrophotometer** measures the amount of light absorbed and transmitted by a dissolved chemical. For solutions we usually refer to the nonabsorbed light as transmitted light rather than reflected light. By measuring the pattern of absorbance or transmittance we can identify a chemical and its concentration.

A spectrophotometer separates white light into a spectrum of colors (wavelengths). It then directs a specific wavelength of light at a tube containing a solution that we are trying to measure. The light is either absorbed by the dissolved substance or transmitted through the solution and exits the sample tube. The spectrophotometer compares the amount of light exiting the tube (that is, the transmitted light) with the amount entering the tube and calculates transmittance—the more solute, the lower the transmittance. The spectrophotometer also calculates the amount of light absorbed—the more solute, the higher the absorbance. The basic parts of a spectrophotometer are shown in figure 8.2.

The **light source** of a spectrophotometer produces white light, a combination of all visible wavelengths. A mixture of all colors of light is white. Spectrophotometry may involve wavelengths outside the visible range, such as ultraviolet and infrared, but the spectrophotometer must have special light sources to produce these wavelengths. In this exercise you will work only with visible light.

The **filter** is adjusted to select the wavelength that you wish to pass through the sample. The filter may be a prism that separates white light into a rainbow of colors and focuses the desired wavelength (color) on the sample. Or, the filter may be a series of colored glass plates that absorbs all but the selected wavelength focused on the sample.

The **sample** is a solution contained in a clear test tube or cuvette made of glass or quartz. Light of the specific wavelength determined by the filter passes into the sample, where it may be completely or partially absorbed or transmitted. The amount of light absorbed depends on the amount and type of chemicals in the solution and the dimensions of the tube.

A **blank** is a test tube or cuvette containing only the solvent used to dissolve the chemical you are analyzing. A blank is used to calibrate the spectrophotometer for the solution used in your experiment. For most of your experiments, the solvent, and therefore the blank, is distilled water.



Figure 8.2 A spectrophotometer and its parts.

Any light transmitted through the sample exits the sample on the opposite side and is focused on a **photodetector** that converts light energy into electrical energy. The amount of electricity produced by the photodetector is proportional to the amount of transmitted light: The more light that is transmitted through the sample, the more electricity produced. The **meter** on the front of the spectrophotometer measures the electrical current produced by the photodetector and displays the results on a scale of absorbance or transmittance values. If a chemical is in solution we usually refer to its transmittance rather than its reflectance, although both terms refer to nonabsorbed light.

The double scale on the spectrophotometer indicates that you can measure either absorbance or transmittance of radiation. **Absorbance** is the amount of radiation retained by the sample, and **transmittance** is the amount of radiation passing through the sample. In mathematical terms, transmittance is the intensity of light exiting the sample divided by the amount entering the sample. Transmittance is usually expressed as a percentage:

$$\textbf{Percentage Transmittance} = (I_t/I_o) \times 100$$

where

I_t = **transmitted (exiting) light intensity**
I_o = **original (entering) light intensity**

Absorbance is the logarithm of the reciprocal of transmittance and is expressed as a ratio with no units:

$$\textbf{Absorbance} = \log_{10} (I_o/I_t)$$

Absorbance is not a percentage and is not simply the opposite (reciprocal) of transmittance. Instead, it is a logarithmic function and has no units. This calculation makes absorbance values directly proportional to the concentration of the substance in solution. Thus, a twofold increase in absorbance indicates a twofold increase in concentration. This convenient and direct relationship between concentration and absorbance helps scientists measure an unknown concentration of a chemical.

To measure unknown concentrations of dissolved chemicals, two procedures must be done—determine a chemical's absorption spectrum and build a standard curve. As you proceed with this exercise be sure that you understand the differences between these procedures.

ABSORPTION SPECTRUM OF COBALT CHLORIDE

Your first task is to learn to operate a spectrophotometer while deriving the **absorption spectrum** of a common chemical, cobalt chloride ($CoCl_2$). The pattern of wavelengths absorbed by $CoCl_2$ is its "fingerprint" because it is unique to that chemical. This fingerprint is the **absorption spectrum** of the chemical and is represented as a graph relating absorbance to wavelength (fig. 8.3). Your instructor may choose to use red dye to simulate $CoCl_2$.



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 8.1 Determine the absorption spectrum of $CoCl_2$

1. **When you get to the lab, turn on the spectrophotometer at your work-station. Let it warm up for 10–15 min before you begin work.**
2. Check with your instructor or manufacturer's directions for any special instructions for using the spectrophotometer in your lab.
3. Prepare seven solutions in spectrophotometer tubes (test tubes or cuvettes) with the mixtures of distilled water and stock solution of $CoCl_2$ (100 mg/mL) listed in table 8.1. Only tubes 0 and 6 are needed to determine the absorption spectrum. You will use the others later in the lab period.
4. After you have prepared the dilutions, clean the outside of all the tubes with a cloth or paper towel.
5. Verify that the solutions in each of your tubes are free of particles (dust, chalk, etc.) that might scatter the light from the spectrophotometer and produce false absorbance values. If necessary, centrifuge the tubes at 2000 rpm for 5–10 min.

Question 2
Why is it important to clean the sample tubes?

6. Cap the tubes and label the caps 0–6. If you label the tube rather than the caps, be sure that the labels don't interfere with light entering the tube while it is in the spectrophotometer.
7. Place the blank (tube 0) in the sample holder of the spectrophotometer.
8. Adjust the filter to the lowest wavelength (350 nm) and read the absorbance value indicated on the meter. The distilled water blank has no color and should not absorb any visible light.
9. If the absorbance is not zero, use the zero adjust knob to calibrate the meter to zero on the absorbance scale.
10. Remove the blank and replace it with tube 6 (50 mg/mL). This is the sample you will use to determine the absorption spectrum of cobalt chloride.
11. After the meter has stabilized (5–10 sec) read the absorbance value and record the wavelength and absorbance value in table 8.2.

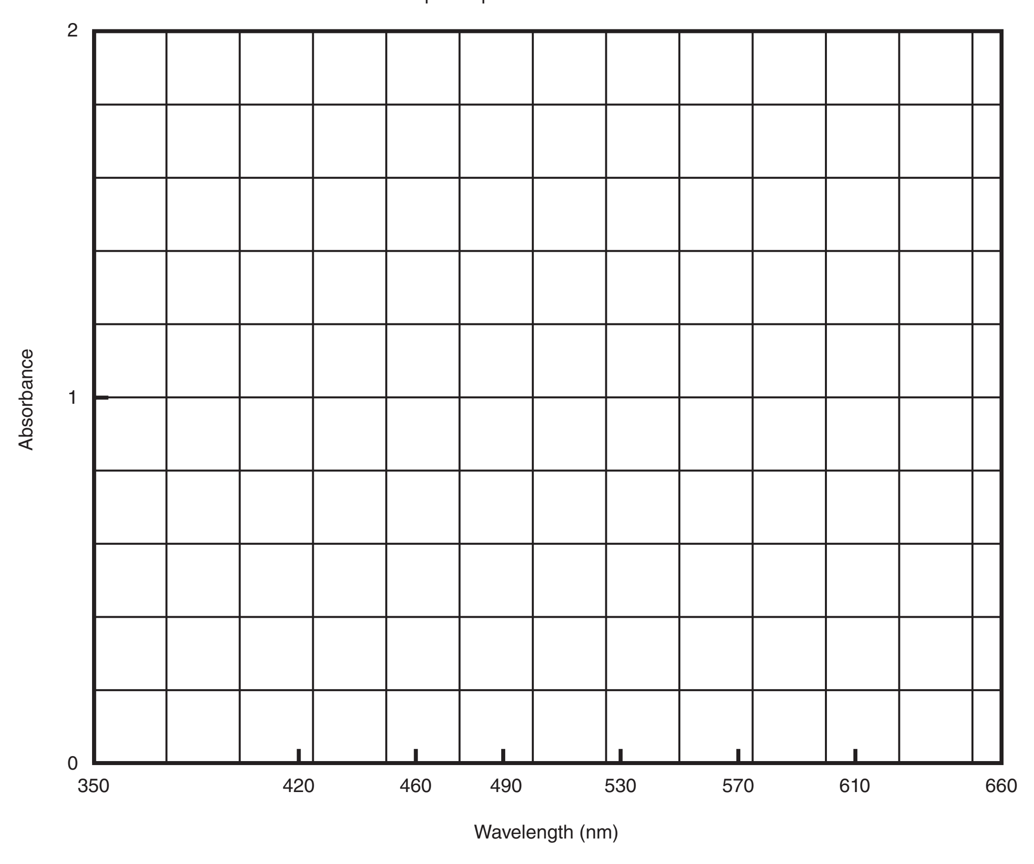


Figure 8.3 Absorption spectrum of cobalt chloride ($CoCl_2$).

TABLE 8.1

VOLUMES OF COBALT CHLORIDE STOCK SOLUTION (100 MG/ML) AND WATER USED TO PREPARE SEVEN KNOWN DILUTIONS

TUBE NUMBER	CONCENTRATION OF $CoCl_2$ (MG/ML)	$CoCl_2$ STOCK (ML)	DISTILLED H_2O (ML)
0	0	0	10.0
1	1	0.1	9.9
2	10	1.0	9.0
3	20	2.0	8.0
4	30	3.0	7.0
5	40	4.0	6.0
6	50	5.0	5.0

12. Remove tube 6 and adjust the filter to 420 nm.

13. Put the blank back into the spectrophotometer and readjust for zero absorbance at the new wavelength. **The spectrophotometer should be recalibrated with the blank often, especially when you change the wavelength.**

14. Insert tube 6 and measure its absorbance at the new wavelength. Record the absorbance in table 8.2.

15. Complete table 8.2 for the other wavelengths by repeating steps 5–12 and measuring the absorbance of the contents of tube 6.

The absorbance values in table 8.2 represent the absorption spectrum for $CoCl_2$ and are expressed best with a graph. Plot on figure 8.3 the *Absorbance* versus *Wavelength* values recorded in table 8.2. Connect the points with straight lines.

Table 8.2

Absorbance for $CoCl_2$ (50 mg/mL)

Wavelength	Absorbance	Wavelength	Absorbance
350 nm	________	530 nm	________
420 nm	________	570 nm	________
460 nm	________	610 nm	________
490 nm	________	660 nm	________

Table 8.3

Absorbance Values for Six Solutions of Known Concentration (Standards) of $CoCl_2$ at the Peak Absorbance Wavelength

Concentration of Standards (mg $CoCl_2$/mL)	Absorbance	Peak Wavelength = ________
1	________	________
10	________	________
20	________	________
30	________	________
40	________	________
50	________	________

Question 3

a. What wavelength is the peak absorbance of $CoCl_2$?

b. Why is it important to recalibrate with your blank sample often?

c. Would you expect a curve of the same shape for another molecule such as chlorophyll? Why or why not?

THE STANDARD CURVE

A graph showing a chemical's concentration versus its absorbance of a wavelength of light is called a **standard curve,** and the relationship is a straight line. In this exercise you will construct a standard curve and then use it to determine some unknown concentrations of solutions of $CoCl_2$ prepared by your instructor. Use the six dilutions that you prepared previously to construct your standard curve for $CoCl_2$. These solutions are **standards** because their concentrations are known, and they are used to determine the concentration of an unknown solution. The absorbance of each standard is measured at the peak wavelength of the absorption spectrum for $CoCl_2$.

Procedure 8.2 **Construct a standard curve for cobalt chloride**

1. Refer to your data in table 8.2. Determine the wavelength of peak absorbance for $CoCl_2$ and set the filter of your spectrophotometer to this wavelength.
2. Insert the solvent blank (tube 0) and adjust the spectrophotometer for zero absorbance.
3. Replace the blank with tube 1 (1 mg $CoCl_2$/mL), measure its absorbance, and then record the absorbance value in table 8.3.
4. Repeat steps 2–3 for the other five tubes (standards). Be sure and check the zero-absorbance calibration with the blank before each standard measurement.
5. To construct your standard curve for $CoCl_2$, plot the data in table 8.3 with *Concentration* (*mg/mL*) on the horizontal axis and *Absorbance* on the vertical axis on the graph paper at the end of this exercise.
6. Because the relationship between concentration and absorbance is linear (directly proportional), you should draw a straight line that lies as close as possible to each

TABLE 8.4

MEASUREMENTS OF ABSORBANCE AND CONCENTRATION FOR FOUR UNKNOWN SOLUTIONS OF $CoCl_2$

Tube Number	Absorbance	Concentration (mg/mL)
Unknown ______	______	______
Unknown ______	______	______
Unknown ______	______	______
Unknown ______	______	______

data point. Do not merely connect the dots. Extremely high concentrations of a solute can produce a nonlinear segment of the standard curve. However, the concentrations used in this lab exercise are not high enough to produce such "saturation" effects.

7. If a computer and software are available, calculate and plot the line of best fit.

Question 4

Do the plotted data points of your standard curve lie on a straight line?

Using the Standard Curve to Measure the Unknown Concentration of a Solution

After you have created a standard curve, measuring the unknown concentration of a $CoCl_2$ solution is easy. Your instructor has prepared a series of numbered tubes containing unknown concentrations of $CoCl_2$.

Procedure 8.3 **Determine unknown concentrations**

1. Obtain a tube with an unknown solution and record the tube number in table 8.4.
2. Use the blank tube (tube 0) to zero the spectrophotometer at the wavelength of peak absorbance for $CoCl_2$.
3. Measure the absorbance of the unknown solution and record this value in table 8.4.
4. Find this absorbance value on the vertical axis of your standard curve and draw a line from this point parallel to the horizontal axis until the line intersects the standard curve (see the example in fig. 8.4).
5. Draw a line from the intersection straight down until it intersects the horizontal axis. This point on the horizontal axis marks the concentration of the unknown solution.
6. Record the concentration of the unknown solution in table 8.4.
7. Obtain three more tubes with unknown solutions, determine their absorbance and concentration, and record the values in table 8.4. Ask your instructor to check your results.

ABSORPTION SPECTRUM OF CHLOROPHYLL

To give you more experience with absorption spectra, your instructor has prepared a plant extract containing chlorophyll, a photosynthetic pigment (fig. 8.5). The extract was made by grinding leaves in acetone.



Acetone is flammable. Keep all solvents away from hotplates and flames at all times.

Procedure 8.4 **Determine the absorption spectrum of chlorophyll**

1. Obtain a tube of the extract and prepare a blank. Your instructor will provide the solvent used for the blank.
2. Using procedure 8.3 for determining an absorption spectrum, measure the absorbance of chlorophyll for at least eight wavelengths available on your spectrophotometer as listed in table 8.5.
3. Record your results in table 8.5.
4. Graph your results (in fig. 8.6) as you did for the absorption spectrum of $CoCl_2$.

Question 5

a. What is the proper blank for determining the absorption of chlorophyll in a plant extract?

b. Which wavelengths are least absorbed by chlorophyll?

c. Which wavelengths are most absorbed by chlorophyll?

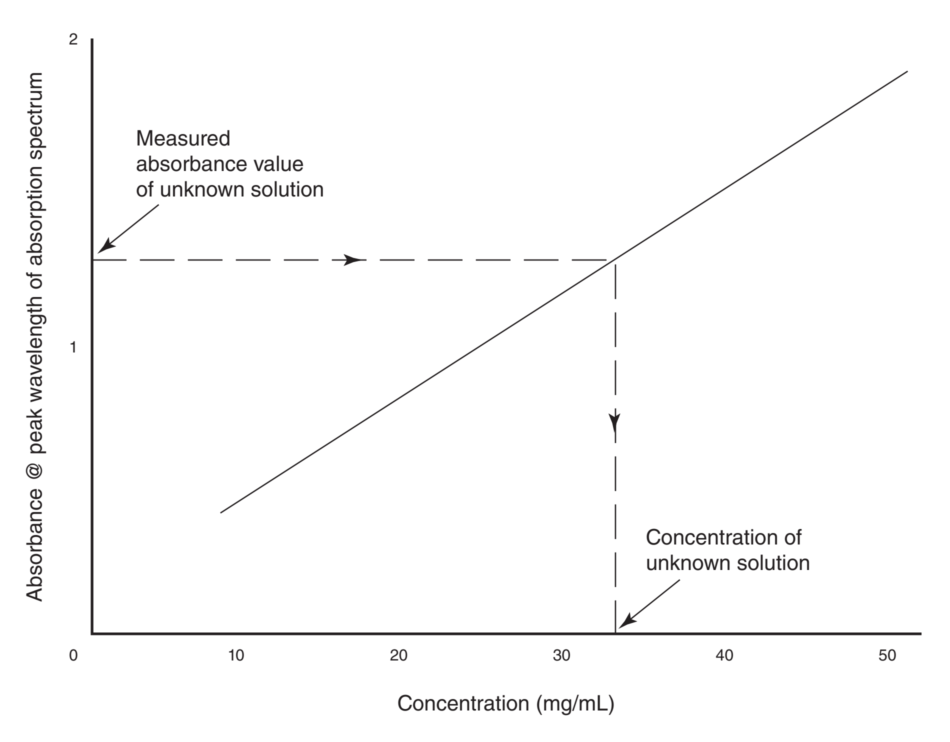


Figure 8.4 A standard curve showing the graphical determination of the concentration of an unknown solution of $CoCl_2$.

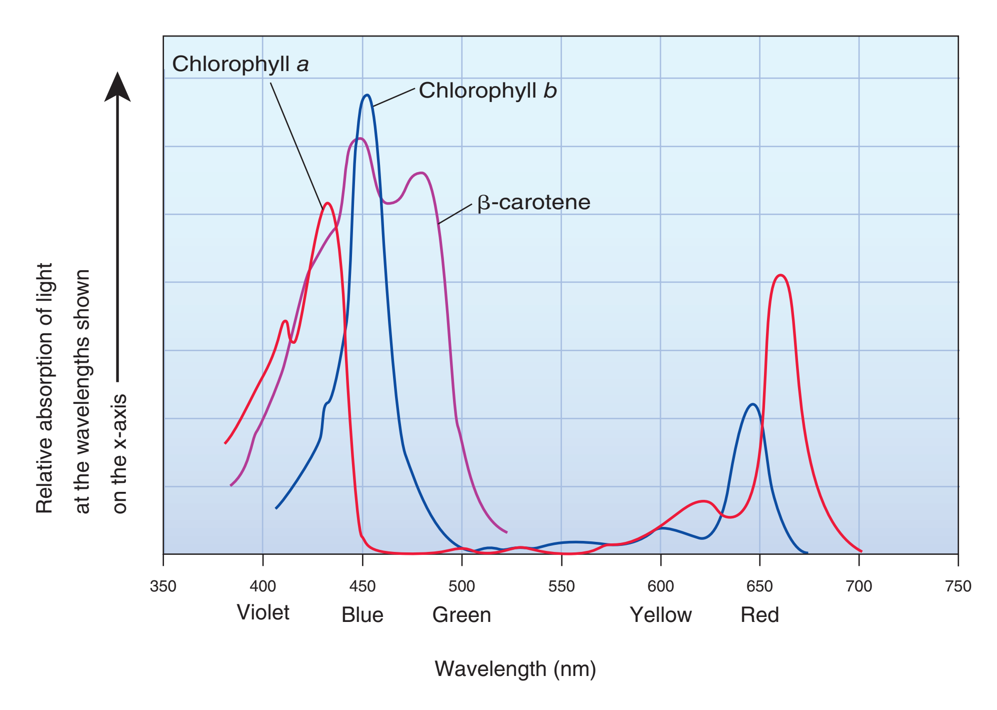


Figure 8.5 The absorption spectrum of chlorophyll and carotenoids. The peaks represent wavelengths of sunlight absorbed by the two common forms of photosynthetic pigment, chlorophylls *a* and *b*, and by the carotenoids. Chlorophylls absorb predominantly violet-blue and red light in two narrow bands of the spectrum and reflect green light in the middle of the spectrum; this is why chlorophyll appears green. Carotenoids absorb mostly blue and green light and reflect orange and yellow light; this is why carotenoids appear orange and yellow.

Table 8.5

Absorbance Values for a Plant Extract Containing Chlorophyll

Wavelength	Absorbance	Wavelength	Absorbance
350 nm	________	530 nm	________
420 nm	________	570 nm	________
460 nm	________	610 nm	________
490 nm	________	660 nm	________

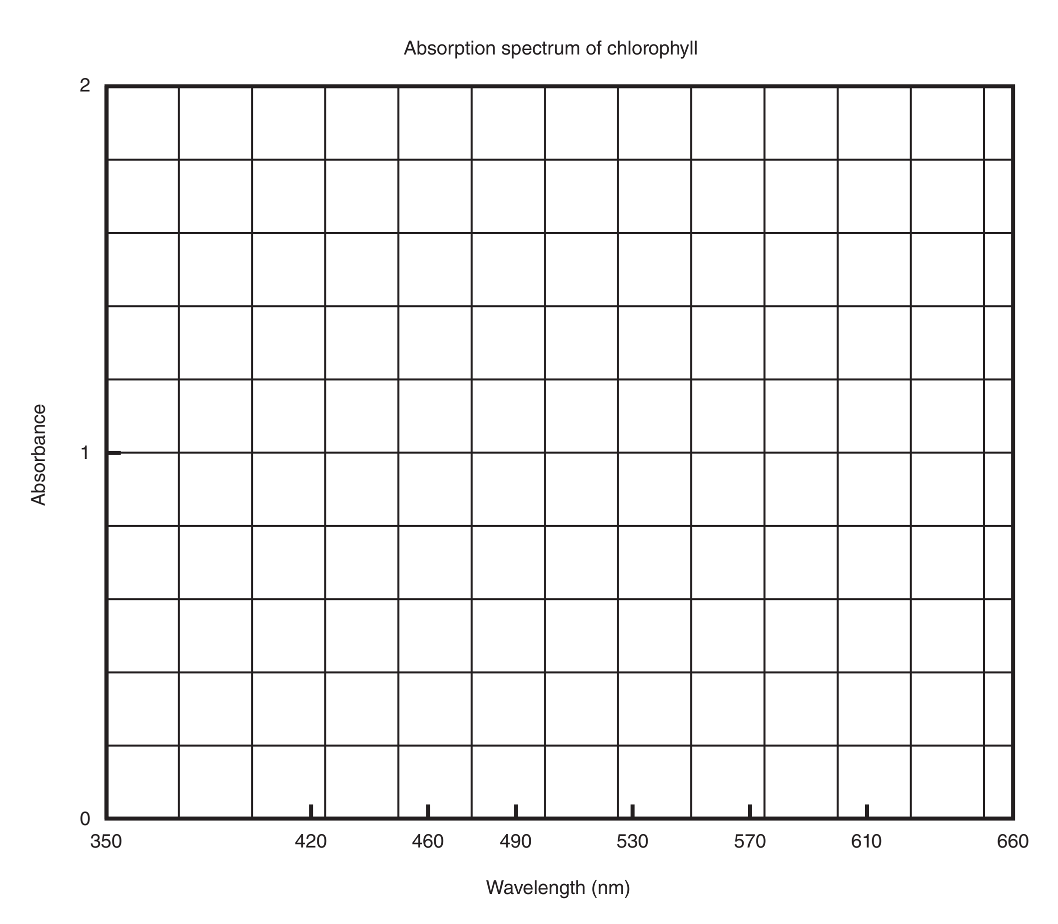


Figure 8.6 Absorption spectrum of chlorophyll.

INVESTIGATION

The Impact of Contaminants on Spectrophotometry

Observation: Spectrophotometry uses the differential absorption of light to identify and measure the concentrations of various molecules. The molecules of interest are not the only molecules that absorb light; contaminants do also and, therefore, affect measurements.

Question: How does a contaminant such as salt affect the absorption of light by a spectrophotometric sample?

a. Establish a working lab group and obtain Investigation Worksheet 8 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 8 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What is the difference between an absorption spectrum and a standard curve?

2. Can spectrophotometry be used to determine the concentration of "colorless" solutes such as salt or sugar? Explain your answer.

3. Why is it important to use standards or to develop a standard curve in spectrophotometry?

4. Why do leaves of plants appear green? Would plants grow well in greenish-yellow light? Explain your answer.

5. How might the basic techniques that you learned about today be used to solve crimes?



Exercise 9

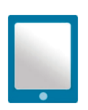
Diffusion and Osmosis

Passive Movement of Molecules in Biological Systems

Learning Objectives

By the end of this exercise you should be able to:

1. Understand Brownian movement and its relationship to molecular movement.
2. Explain the factors controlling a substance's direction and rate of diffusion.
3. Determine the direction and relative rates of diffusion of molecules of different sizes.
4. Predict the direction and rate of osmosis into and out of cells surrounded by hypotonic, hypertonic, and isotonic environments.
5. Describe how hypotonic, hypertonic, and isotonic solutions affect the volume and integrity of blood cells and plant cells.



Please visit connect.mheducation.com to review online resources tailored to this lab.

All molecules display random thermal motion, or kinetic energy; this is why a dissolved molecule tends to move around in a solution. Kinetic energy causes molecules to diffuse outward from high concentrations to lower concentrations. This random movement is constant, but the net movement of molecules from areas of high concentration to areas of low concentration continues until the distribution of molecules is homogenous throughout the solution. For example, when a dye dissolves in a container of water, the dye disperses. The rate of dispersal depends on the temperature and concentration of the dye, the size of the dye molecules, and the temperature and the density of the solvent. Regardless of this rate, the dye will eventually disperse itself uniformly throughout the solution. This is easily illustrated by placing a drop or crystal of dye into a glass of water (fig. 9.1).

BROWNIAN MOVEMENT

Heat causes **random motion** of molecules that passively moves molecules in biological systems. Although we cannot directly see molecules move, we can see small particles move after they collide with moving molecules. This motion was originally described in 1827 by Robert Browning after he prepared dead pollen grains in water and viewed them with a microscope. The dead pollen grains were moving. They were being jostled by collisions from water molecules. **Brownian movement** is visible using your microscope's high magnification. Carmine red dye mixed with soap is a good suspension of small particles. The red dye particles are small enough to vibrate when water molecules bump into them.



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 9.1 **Observe Brownian movement**

1. Place a small drop of a carmine red suspension on a microscope slide and cover the drop with a coverslip.
2. Focus first at low magnification; then rotate to higher power (40×). Be careful not to get dye on the objective lens.



Figure 9.1 Beakers of water before and after diffusion of a dye. Random movements of water and dye molecules drive diffusion, eventually resulting in a uniform distribution of the dye. Convection currents may also help distribute the dye in these solutions.

3. Fine focus the image. At first the field of view will appear uniformly reddish gray. But with sharp focus, you will see thousands of small particles vibrating rapidly.
4. Check with your instructor to determine if your microscope has oil immersion magnification and if you need this to easily view the particles. If needed, follow their instructions for using this objective.
5. Leave the microscope light on. Observe any changes in motion caused by increased heat.

Question 1

a. Briefly describe your observation of the moving pigment particles.

b. Does the movement of particles change visibly with heat? If so, how?

DIFFUSION

In biological systems, substances often move through solutions and across membranes in a predictable direction. This passive, directional movement of molecules is **diffusion** (fig. 9.2). The *direction* of diffusion depends on the concentration gradient, heat, and pressure. Specifically, molecules diffuse from an area of high concentration, heat, and pressure to an area of low concentration, heat, and pressure. The *rate* of diffusion is determined by the steepness of the gradient and other characteristics of the specific molecule in question, such as its size, polarity, or solubility.

Temperature, pressure, and concentration all affect diffusion, but temperature and pressure are relatively constant in most biological systems. Therefore, concentration is usually the best predictor of a substance's direction of diffusion. But remember that temperature and pressure gradients may also affect diffusion.

Diffusion and Molecular Weight

Before your class meeting, your instructor inoculated some petri plates containing agar with either potassium permanganate (molecular weight = 158 g mole^{-1}), malachite green (molecular weight = 929 g mole^{-1}), or methylene blue (molecular weight = 374 g mole^{-1}).

Question 2

Which would you predict would diffuse faster: a substance having a high molecular weight or a substance having a low molecular weight? Why?

Procedure 9.2 **Observe diffusion as affected by molecular weight**

1. Examine one of the prepared agar plates and note the three halos of color. These halos indicate that the chemicals have diffused away from the two original spots and moved through the agar.
2. Measure the halos with a ruler.

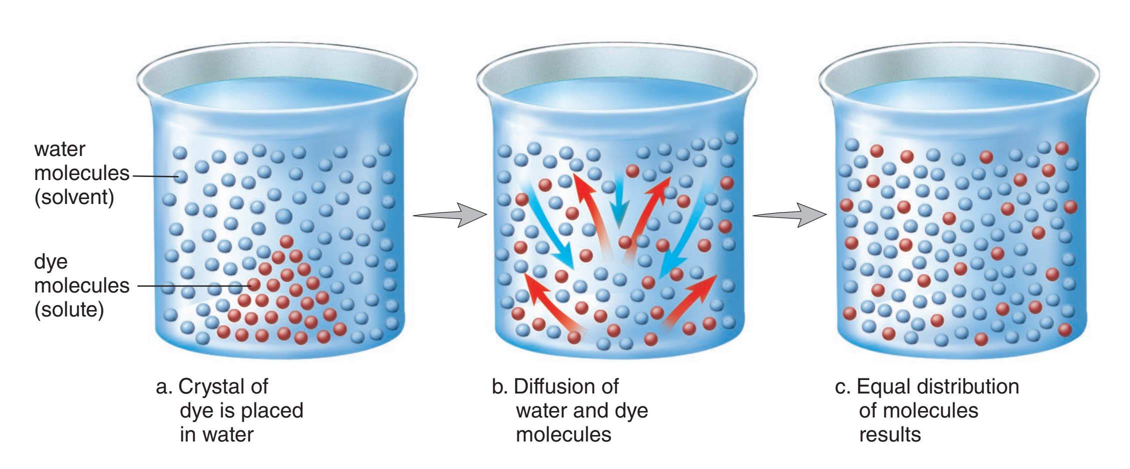
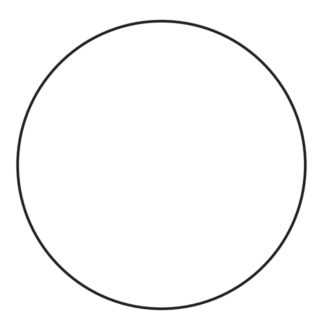


Figure 9.2 Process of diffusion. Diffusion is spontaneous, and no chemical energy is required to bring it about. (*a*) When a dye crystal is placed in water, it is concentrated in one area. (*b*) The dye dissolves in the water, and there is a net movement of dye molecules from higher to lower concentration. There is also a net movement of water molecules from a higher to a lower concentration. (*c*) Eventually the water and dye molecules are equally distributed throughout the container.

3. Record within the outline of a petri dish your observations of the size of each halo.



Question 3

a. Considering the different molecular weights of potassium permanganate, malachite green, and methylene blue, which should have the larger halo after the same amount of time? Why?

b. Do molecules stop moving when diffusion stops? Explain your answer.

DIFFUSION AND DIFFERENTIALLY PERMEABLE MEMBRANES

Membranes surround cells and organelles and organize an immense number of simultaneous reactions. However, the barrier imposed by a cellular membrane does not isolate a cell. Instead, it allows a cell to *selectively* communicate with its environment. Membranes are "alive" in the sense that they respond to their environment and allow some molecules to pass while retarding others. Thus, membranes are selective and **differentially permeable** (fig. 9.3). This selective permeability results from the basic structure of membranes. They have a two-layered core of nonpolar lipid molecules that selects against molecules not readily soluble in lipids. You'll learn more about membrane structure in Exercise 10.

Membrane permeability to a solute depends on the solute's size, charge (ions), polarity, and lipid solubility. **Polar molecules** have positively charged areas and negatively charged areas. **Nonpolar molecules** have no local areas of charge. Small, uncharged, nonpolar, lipid-soluble molecules pass most easily through the lipid core of a membrane (see fig. 10.4).

In general, small molecules pass through a membrane more easily than do large molecules. We can demonstrate membrane selection for molecular size by using a bag made from **dialysis tubing** to model a differentially permeable membrane. **Dialysis** is the separation of dissolved substances by means of their unequal diffusion through a differentially permeable membrane. Dialysis

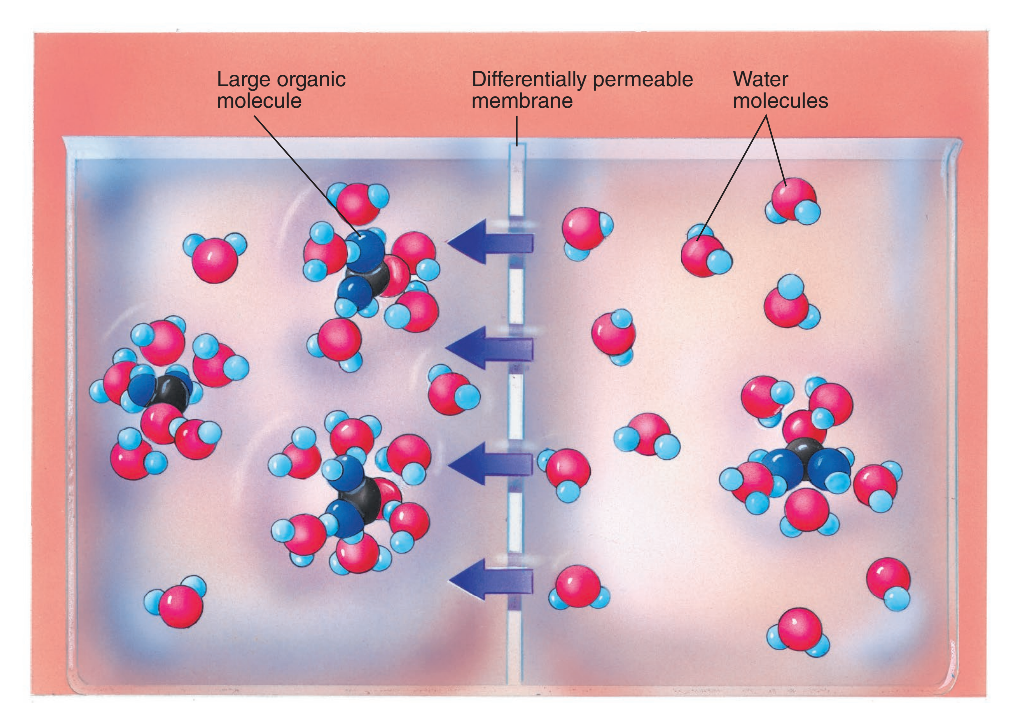


Figure 9.3 A differentially permeable membrane prevents the movements of some molecules but not others. Arrows indicate the movement of small molecules, such as water, from an area of high concentration to an area of lower concentration. The large molecules cannot pass through the membrane.

membranes (or tubing) are good models of differentially permeable membranes because they have small pores that allow small molecules such as water to pass, but block large molecules such as glucose. However, remember that living cell membranes also discriminate among molecules based on charge and solubility, whereas dialysis tubing used in these procedures does not. Dialysis tubing is only a physical model of a cell, and its selectivity is based only on molecular size.

Examine some dialysis tubing. Although the dried material looks like a narrow sheet of cellophane, it is a flattened, open-ended tube.

In procedure 9.3 you will use two indicators: **phenolphthalein** and **iodine.** Phenolphthalein is a pH indicator that turns red in basic solutions (see Exercise 5). Iodine is a starch indicator that changes from yellow to dark blue in the presence of starch (see Exercise 6).

Procedure 9.3 Observe diffusion across a differentially permeable membrane

1. Obtain four pieces of string or dialysis clips and two pieces of water-soaked dialysis tubing approximately 15 cm long.

2. Seal one end of each bag by folding over 1–2 cm of the end. Then accordion-fold this end and tie it tightly with monofilament line or string (fig. 9.4). The ends of the tube must be sealed tightly to prevent leaks.

3. Roll the untied end of each tube between your thumb and finger to open it and form a bag.

4. Use either a graduated cylinder or pipet to fill one tube with 10 mL of water and add three drops of phenolphthalein. Seal the open end of the bag by folding the end and tying it securely.

5. Fill the other bag with 10 mL of starch suspension. Seal the open end of the bag by folding the end and tying it securely.

6. Gently rinse the outside of each bag in tap water.

7. Fill a beaker with 200 mL of tap water and add 10 drops of 1 M sodium hydroxide (NaOH). Submerge the dialysis bag containing phenolphthalein in the beaker.



Do not spill the NaOH. It is extremely caustic.

8. Fill a beaker with 200 mL of tap water and add 20–40 drops of iodine. Submerge the dialysis bag containing starch in the beaker.

9. Observe color changes in the two bags' contents and the surrounding solutions.

10. In this experiment some of the solutes can move through the membrane and some cannot. Water can freely move through the membrane, but the movement of water is not of interest in this experiment.

11. Record in figure 9.5 the color inside and outside the bags. Label the contents inside and outside the bags.

Question 4

a. Describe color changes in the two bags and their surrounding solutions.

b. For which molecules and ions (phenolphthalein, iodine, starch, Na^+, OH^-) does your experiment give evidence for passage through the semipermeable membrane?

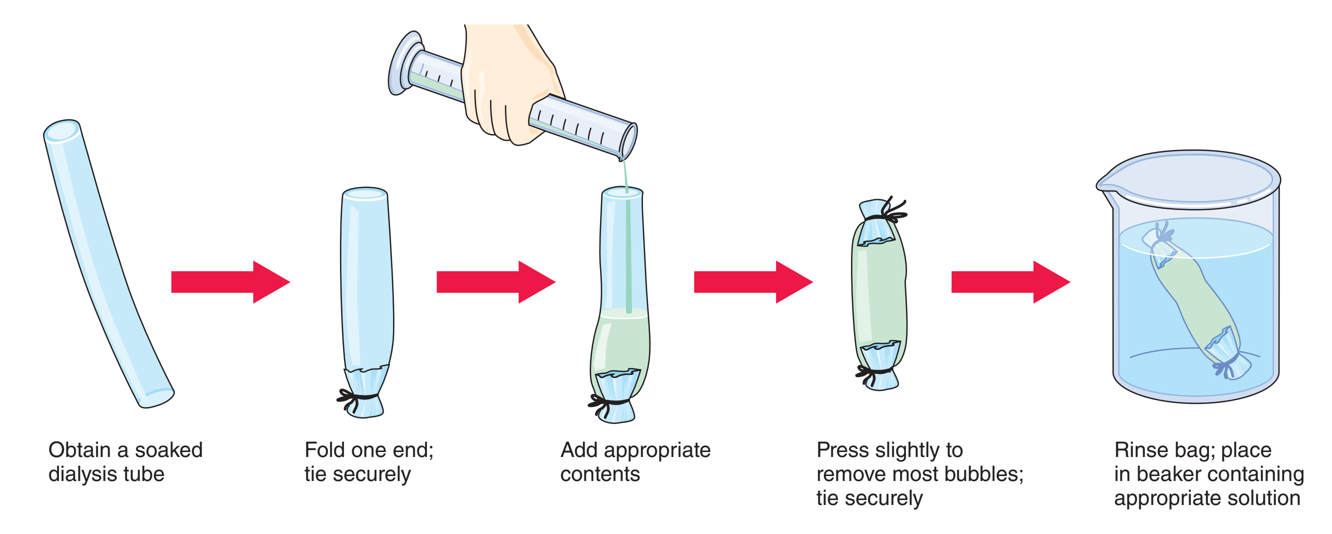
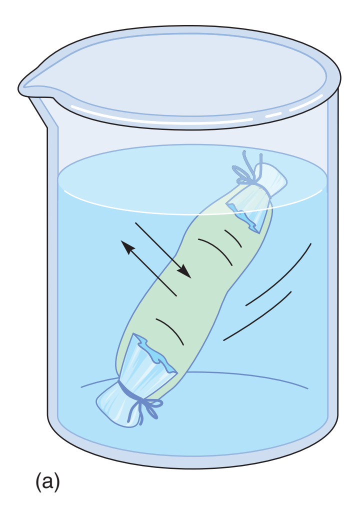


Figure 9.4 Preparation of dialysis tubing as a model of a cell surrounded by a differentially permeable membrane.



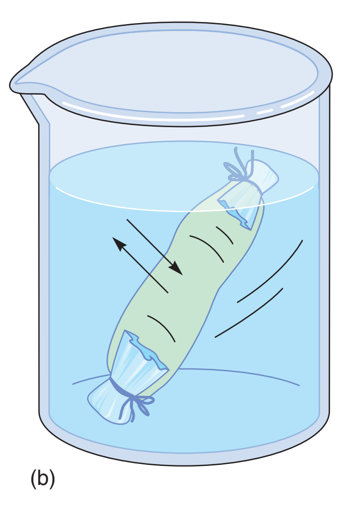


Figure 9.5 (*a*) Movements and reaction of sodium hydroxide and phenolphthalein through a differentially permeable membrane. (*b*) Movements and reaction of iodine and starch through a differentially permeable membrane. Record the results of your experiment on this diagram.

c. What characteristic distinguishes those molecules and ions passing through the membrane from those that do not pass through the membrane?

OSMOSIS AND THE RATE OF DIFFUSION ALONG A CONCENTRATION GRADIENT

The speed at which a substance diffuses from one area to another depends primarily on the concentration gradient between those areas. For example, if concentrations of a diffusing substance at the two areas differ greatly, then diffusion is rapid. Conversely, when the concentration of a substance at the two areas is equal, the diffusion rate is zero and there is no net movement of the substance.

Osmosis is diffusion of water across a differentially permeable membrane. Osmosis follows the same laws as diffusion but always refers to water, the principal solvent in cells. A **solution** is a homogenous, liquid mixture of two or more kinds of molecules. A **solvent** is a fluid that dissolves substances, and a **solute** is a substance dissolved in a solution.

We can simulate osmosis by using dialysis bags to model cells under different conditions and measuring the direction and rate of osmosis. Each of the four dialysis bags in the following experiment is a model of a cell. Bag A simulates a cell containing a solute concentration that is hypotonic relative to its environment. **Hypotonic** describes a solution with a lower concentration of solutes, especially those solutes that do not pass across the surrounding membrane. Water moves across semipermeable membranes out of hypotonic solutions. Conversely, the solution surrounding bag A is hypertonic relative to the cell. **Hypertonic** refers to a solution with a high concentration of solutes.

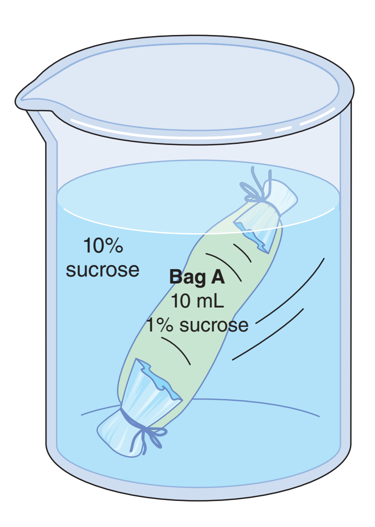
Bag B represents a cell whose solute concentration equals the concentration in the environment; this cell (bag B) is isotonic to its environment. **Isotonic** refers to two solutions that have equal concentrations of solutes. Bags C and D are both hypertonic to their environment and have higher solute concentrations than the surrounding environment. Remember that the solute (sugar) does not pass through the membrane—only the water does.

NOTE

Start this experiment at the beginning of the lab period so that you'll have enough time to see results.

Procedure 9.4 **Observe osmosis across a concentration gradient**

1. Obtain eight pieces of string and four pieces of water-soaked dialysis tubing each 15 cm long. Seal one end of each tube by folding and tying it tightly.
2. Open the other end of the tube by rolling it between your thumb and finger.
3. Fill the bags with the contents shown in figure 9.6. To label each bag, insert a small piece of paper with the appropriate letter (A, B, C, or D written on it in pencil).
4. For each bag, loosely fold the open end and press on the sides to push the fluid up slightly and remove most of the air bubbles. Tie the folded ends securely, rinse the bags, and check for leaks.
5. Gently blot excess water from the outside of the bags and weigh each bag to the nearest 0.1 g.
6. Record these initial weights in table 9.1 in the first column.
7. Place bags B, C, and D in three individual beakers or one large bowl filled with 1% sucrose (fig. 9.6). Record the time.



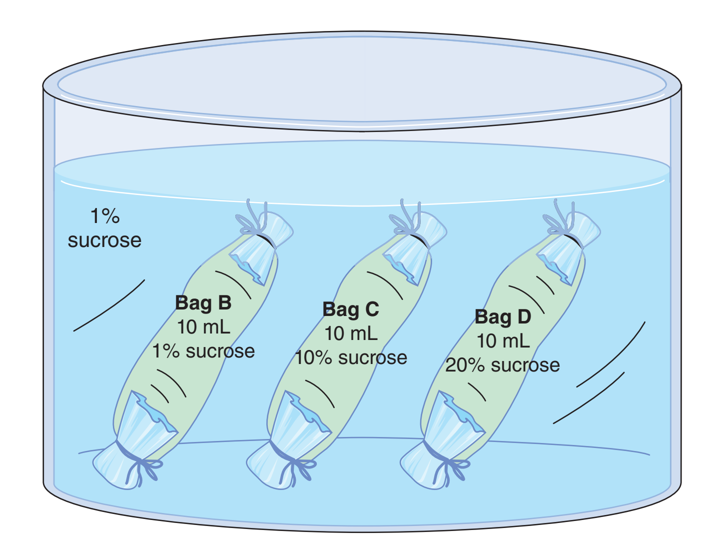


Figure 9.6 Experimental setup for four cellular models used to measure the rate of osmosis.

8. Place bag A in a 250-mL beaker and fill the beaker with 150 mL of 10% sucrose. Record the time.
9. Remove the bags from the beakers at 15-min intervals for the next hour (or at intervals indicated by your instructor), gently blot them dry, and weigh them to the nearest 0.1 g. Handle the bags delicately to avoid leaks, and quickly return the bags to their respective containers.
10. During the 15-min intervals, use your knowledge of osmosis to make hypotheses about the direction of water flow in each system (i.e., into or out of bag), and the extent of water flow in each system (i.e., in which system will osmosis be most rapid?).
11. For each 15-min interval record the total weight of each bag and its contents in table 9.1. Then calculate and record in table 9.1 the change in weight since the previous weighing.

Procedure 9.5 Graph osmosis

1. Use the graph paper at the end of this exercise to construct a graph with *Total Weight (g)* versus *Time (min)*. *Total Weight* changed in response to differences in the independent variable, so *Total Weight* is the **dependent variable.** The dependent variable is always graphed on the vertical axis. *Time* is the variable that you established and actively controlled and, therefore, is the **independent variable.** The independent variable is always graphed on the horizontal axis.
2. Graphs must have a title (e.g., Relationship between Time and Weight Gain), correctly labeled axes (e.g., *Total Weight*, *Time*), a label showing measurement units (e.g., g and min), and values along each axis (e.g., 0, 15, 30, 45, 60). Include these in your graph.
3. Plot the data for total weight at each time interval from table 9.1.
4. Include the data for all four bags as four separate curves on the same graph.

Question 5

a. Did water move across the membrane in all bags containing solutions of sugar?

Table 9.1

Changes in Weight of Dialysis Bags Used as Cellular Models*

	0 Min	15 Min		30 Min		45 Min		60 Min	
	Initial Weight	Total Weight	Change in Weight	Total Weight	Change in Weight	Total Weight	Change in Weight	Total Weight	Change in Weight
Bag A									
Bag B									
Bag C									
Bag D									

*Each change in weight is only for the previous 15-min interval.

b. In which bags did osmosis occur?

c. A concentration gradient for water must be present in cells for osmosis to occur. Which bag represented the steepest concentration gradient relative to its surrounding environment?

d. The steepest gradient should result in the highest rate of diffusion. Examine the data in table 9.1 for Change in Weight during the 15- and 30-min intervals. Did the greatest changes in weight occur in cells with the steepest concentration gradients? Why or why not?

Question 6

a. Refer to your graph. How does the slope of a segment of a curve relate to the rate of diffusion?

b. What influence on diffusion (i.e., temperature, pressure, concentration) causes the curves for bags C and D eventually to become horizontal (i.e., have a slope = 0)?

WATER POTENTIAL

Plants must balance the uptake and loss of water as it moves from one part of a plant to another and in and out of cells by osmosis. However, the concentration gradient of water and solutes doesn't solely determine the direction and rate of water movement. Physical pressure influenced by cell walls and evaporation is also important. Plant physiologists refer to the combined effects of these factors as **water potential;** water flows from an area of high water potential to an area of low potential. Both high water concentration (low solute concentration) and high pressure increase water potential. Similarly, high solutes and low pressure decrease water potential. In simple terms, water flows through a plant from higher water potentials of roots toward lower water potentials of leaves. These lower potentials in leaves are created by their loss of water to the atmosphere (see Exercise 33). In the following procedure you will measure the concentration of solutes in potato cells and relate this concentration to water potential.

Procedure 9.6 **Determine the concentration of solutes in living plant cells**

1. Locate the five beakers prepared by your instructor with five concentrations of salt (NaCl) solution.
2. The cylinders of potato that you see in the solutions were all originally the same size (i.e., the same length or weight). Check the beaker labels to determine which measure of size (length or weight) you will be using as your data.
3. Record the initial values in table 9.2.
4. Carefully remove three of the potato cylinders from each solution and measure their size.
5. Record your data in table 9.2.
6. Calculate the mean change in size and record the data in table 9.2.
7. Your instructor may ask you to graph your data (see Question 7f). Follow his or her instructions.

TABLE 9.2

CHANGE IN LENGTH OF POTATO CYLINDERS SURROUNDED BY DIFFERENT SALT CONCENTRATIONS

CONCENTRATION OF SALT SOLUTION (%)	INITIAL SIZE OF CYLINDERS (MILLIMETERS OR GRAMS)	CHANGES IN SIZE OF THREE SAMPLE CYLINDERS			MEAN CHANGE IN SIZE
0	________	________	________	________	________
0.9	________	________	________	________	________
5	________	________	________	________	________
10	________	________	________	________	________
15	________	________	________	________	________

Question 7

a. Which potato cylinders increased in size or weight? Why?

b. Which solution(s) contained a higher concentration of solutes and therefore a lower water potential than in the potato cells? Explain your answer.

c. Which salt solution best approximated the water potential in the potato cells? How do you know this?

d. For a growing potato plant what would you predict as the water potential of the potato relative to the soil? Relative to the leaves?

e. What might be some sources of error in this experiment?

f. How could a graph of your data help you estimate the solute concentration of potato cells?

HEMOLYSIS OF BLOOD CELLS

Living red blood cells (erythrocytes) are good models for studying osmosis and diffusion in hypotonic, hypertonic, and isotonic solutions. Osmosis occurs when living cells are placed in a hypotonic or hypertonic environment and water diffuses into or out of the cell (fig. 9.7). For example, in the previous experiment, water moved into cells toward the low concentration of water. However, osmosis into animal cells increases the hydrostatic (i.e., water) pressure and may burst the cells because they lack cell walls. This destruction of a cell by the influx of water (causing the cell to burst) is called **lysis.** Such destruction of a red blood cell is called **hemolysis.** If water flows out of a cell into a hypertonic solution, the cell will shrivel and become crenate.

Procedure 9.7 **Observe hemolysis**

1. Obtain and label three test tubes and fill them with the solutions listed in table 9.3.
2. Add four drops of fresh sheep's blood to each tube.



Wash your hands thoroughly after working with blood products. Always handle sheep blood with caution and avoid skin contact.

3. Cover each tube with Parafilm and invert the tubes to mix the contents.
4. Hold each tube in front of a printed page and determine if you can read the print through the solution (fig. 9.8). Record your results in table 9.3.
5. Obtain a microscope, slide, and coverslip.

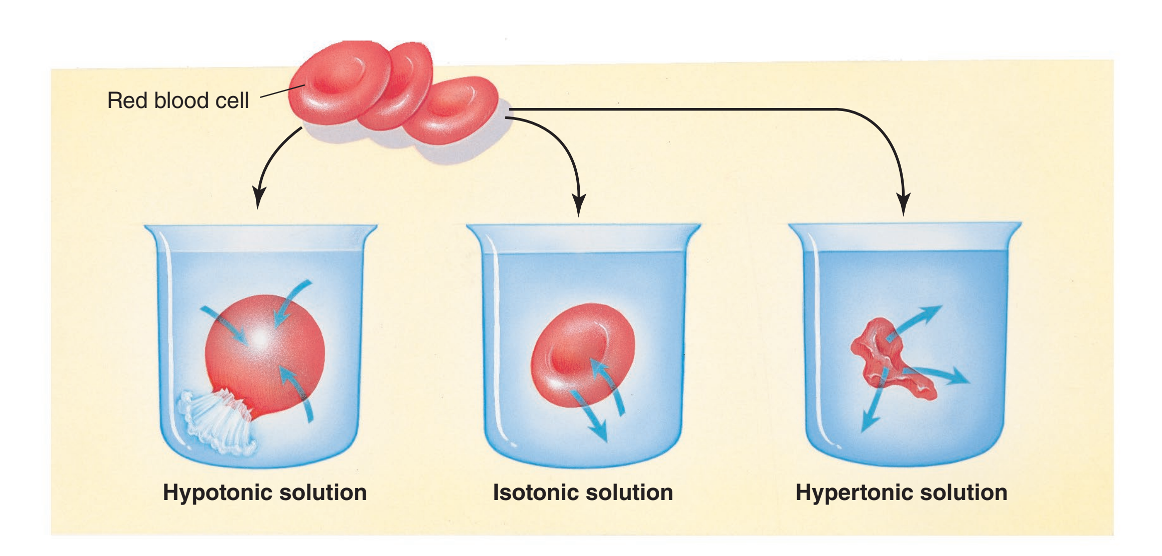


Figure 9.7 Osmosis of water surrounding animal cells. When the outer solution is hypotonic with respect to the cell, water will move into the cells and the cells will lyse; when it is hypertonic, water will move out of the cells and the cells will shrink (i.e., become crenate).

Table 9.3

Hemolysis of Red Blood Cells Exposed to Three Solutions with Different Solute Concentrations

Tube	Contents	Readable Print (yes/no)	Cell Condition (crenate/normal/lysed)
1	5 mL 10% NaCl	________	________
2	5 mL 0.9% NaCl	________	________
3	5 mL distilled water	________	________

6. Use an eyedropper or pipet to obtain one drop from each tube. Make a wet mount and examine the blood cells. Use low magnification first and then higher magnification.

7. Record in table 9.3 the cell's condition as crenate, normal, or lysed.

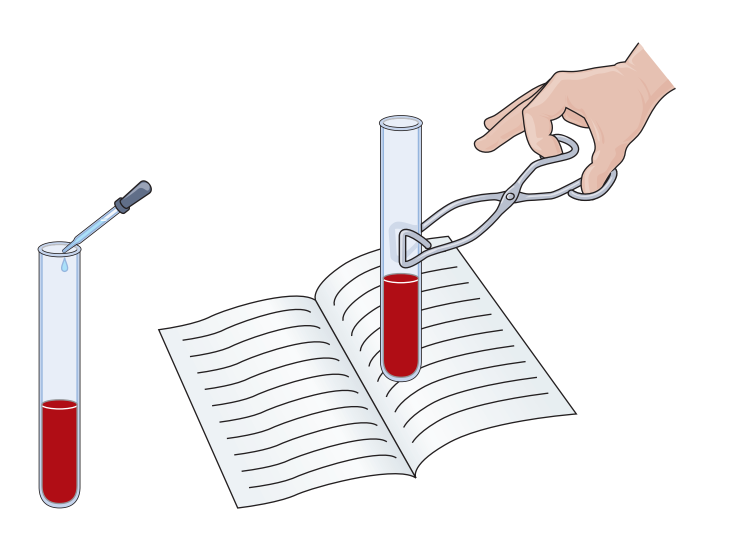


Figure 9.8 Experimental setup for determining hemolysis. Hypotonic solutions will hemolyze cells.

Question 8

a. Through which test tubes could you read the printed page? Why?

b. Which concentration of NaCl lysed the cells?

c. Which of the three solutions most closely approximates the solute concentration in a red blood cell? How do you know?

PLASMOLYSIS OF PLANT CELLS

Plasmolysis is the shrinking of the cytoplasm of a plant cell in response to diffusion of water out of the cell and into a hypertonic solution (high salt concentration) surrounding the cell (fig. 9.9). During plasmolysis the cellular membrane pulls away from the cell wall (fig. 9.10).

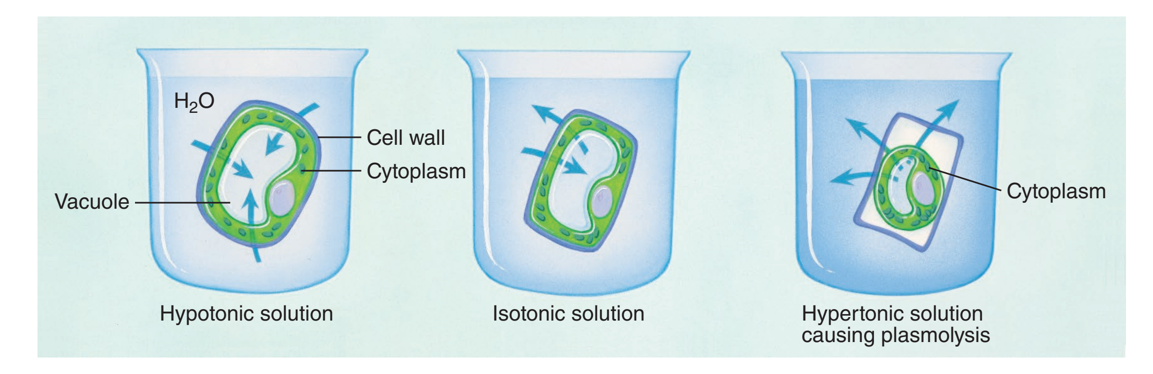
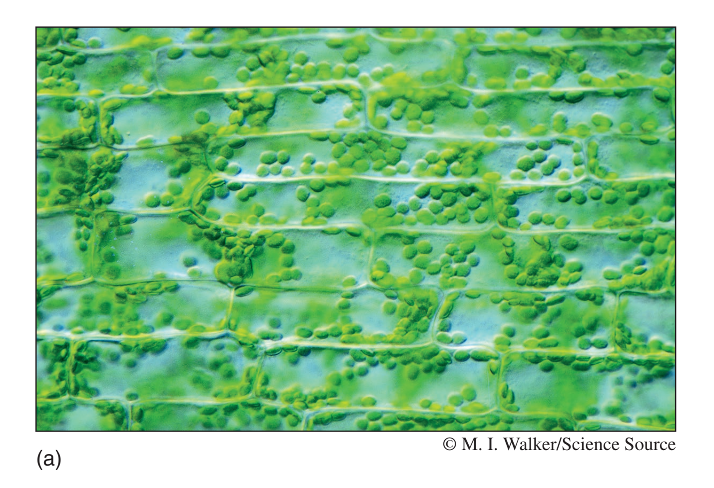


Figure 9.9 Osmosis of water into and out of plant cells. In most plant cells the large central vacuole contains a high concentration of solutes (i.e., the environment surrounding the cell is hypotonic to the cell), so water tends to diffuse into the cells, causing the cells to swell outward against their rigid cell walls. However, if a plant cell is immersed in a high-solute (hypertonic) solution, water will leave the cell, causing the cytoplasm to shrink and pull away from the cell wall.



(a) © M. I. Walker/Science Source



(b) © Ed Reschke/Getty Images

Figure 9.10 (*a*) Turgid *Elodea* cells (100×). (*b*) Plasmolyzed *Elodea* cells (200×) showing the effects of exposure to a hypertonic solution.

Procedure 9.8 Observe plasmolysis

1. Prepare a wet mount of a thin layer of onion epidermis or *Elodea* leaf. Examine the cells.
2. Add two or three drops of 30% NaCl to one edge of the coverslip.
3. Wick this salt solution under the coverslip by touching a piece of absorbent paper towel to the fluid at the opposite edge of the coverslip.
4. Examine the cells. The cytoplasm is no longer pressed against the cell wall. This shrinkage is **plasmolysis.**

Question 9

a. Why did the plant cells plasmolyze when immersed in a hypertonic solution?

b. What can you conclude about the permeability of the cell membrane (i.e., the membrane surrounding the cytoplasm) and vacuolar membrane (the membrane surrounding the vacuole) to water?

To observe the effects of cellular plasmolysis on a larger scale, compare petioles of celery that have been immersed overnight in distilled water or in a salt solution.

Question 10

What causes crispness (i.e., firmness, crunchiness) in celery?

INVESTIGATION

Determining the Concentrations of Solutes in Plant Tissue

Observation: Water moves into and out of cells along a concentration gradient. The more solutes present in cells, the greater the tendency for water to move into the cells.

Question: What is the approximate concentration of solutes in a piece of apple?

a. Establish a working lab group and obtain Investigation Worksheet 9 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 9 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

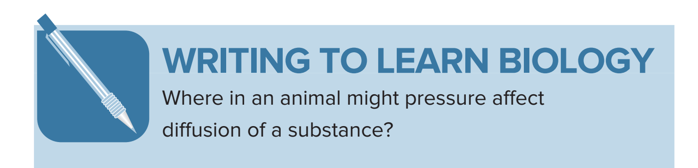
1. Why must particles be extremely small to demonstrate Brownian movement?

2. What is the difference between molecular motion and diffusion?

3. If you immerse your hand in distilled water for 15 min, will your cells lyse? Why or why not?

4. Your data for diffusion of water across a differentially permeable membrane in response to a sucrose gradient could be graphed with *Change in Weight* on the vertical axis rather than *Total Weight*. How would you interpret the slope of the curves produced when you do this?

5. How do cells such as algae and protists avoid lysis in fresh water?



Cellular Membranes

Effects of Physical and Chemical Stress

Learning Objectives

By the end of this exercise you should be able to:

1. Relate membrane structure to its function.
2. Describe the aspects of membrane structure most vulnerable to physical and chemical stress.
3. Predict the effect of common organic solvents and extreme temperatures on membrane integrity.
4. Relate the results of experiments with beet membranes to the general structure and function of membranes.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Membranes separate and organize the myriad of reactions within cells and allow communication with the surrounding environment. Although they are only a few molecules thick (6–10 nm), membranes (1) retard diffusion of selected molecules; (2) house receptor molecules that detect other cells, organelles, and hormones; (3) provide sites for active and passive transport of selected molecules; (4) organize life processes by providing surfaces to accommodate chemical reactions; and (5) help maintain the integrity of cells.

As with all biological entities, the structure of a membrane reflects its function. Membranes consist of a phospholipid bilayer; attached to or embedded within this bilayer are thousands of proteins. A phospholipid molecule consists of a phosphate group and two fatty acids bonded to a three-carbon, glycerol chain (fig. 10.1). Phospholipids have an unevenly distributed charge; that is, they have charged (polar) and uncharged (nonpolar) areas. In phospholipids the phosphate group and glycerol are polar and **hydrophilic** ("water-loving"), whereas the fatty-acid chains are nonpolar and **hydrophobic** ("water-fearing"). Such molecules with two different affinities are **amphipathic,** and amphipathic phospholipids have a natural tendency to self-assemble into a double-layered sheet (fig. 10.2). In this double layer, the hydrophobic tails of lipids form the core of the membrane, and the hydrophilic phosphate groups line both surfaces. This elegant assembly is stable, self-repairing, and resists penetration by most hydrophilic molecules.

Membranes also include proteins dispersed throughout the fluid bilayer of lipids (fig. 10.3). These proteins are not fixed in position; they move about freely and may be densely packed in some membranes and sparse in others. Carbohydrate chains (strings of sugar molecules) are often bound to these proteins and to lipids. These chains serve as distinctive tags that identify particular types of cells. These elaborate molecular elements form the **fluid mosaic model** of membrane structure.

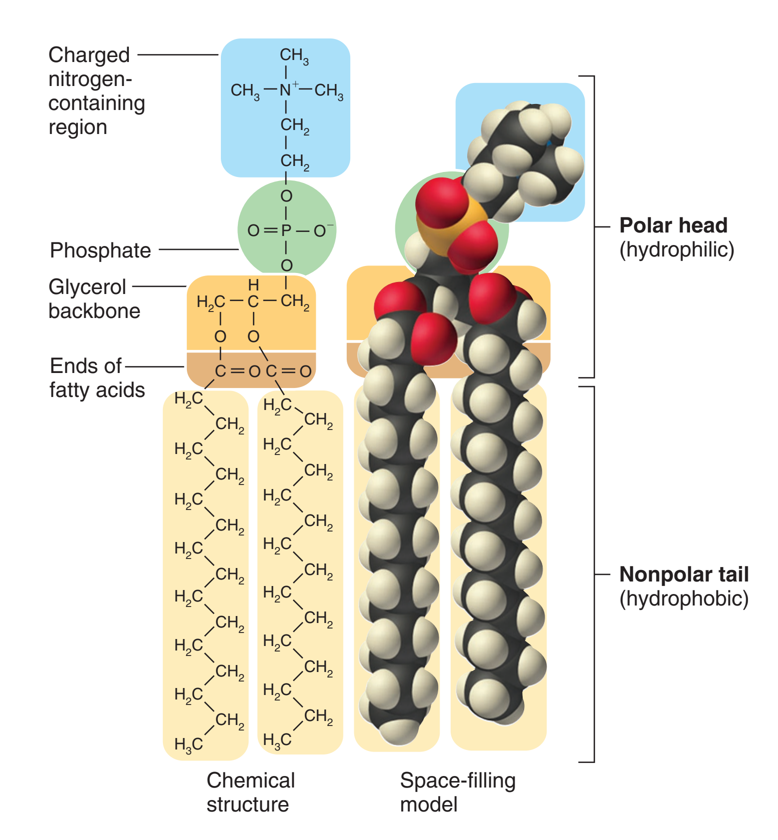


Figure 10.1 The structure of a phospholipid. The backbone of a phospholipid is glycerol, a three-carbon alcohol. Glycerol is bonded to two fatty acids, both hydrophobic, and to one phosphate group, which is hydrophilic. Phospholipids vary by their fatty acids and by the side chains attached to the phosphate. The polar head may include glycerol, sugars, and nitrogen-containing groups as shown here.

Membranes are selectively permeable. The proteins embedded in the phospholipid bilayer can selectively take up or expel molecules that otherwise could not penetrate the membrane. In doing so, these proteins function as pores, permitting and often facilitating the passage of specific ions and polar molecules. In addition to forming pores and sites for active transport, membrane-bound proteins also function as enzymes and receptors that detect signals from the environment or from other cells.

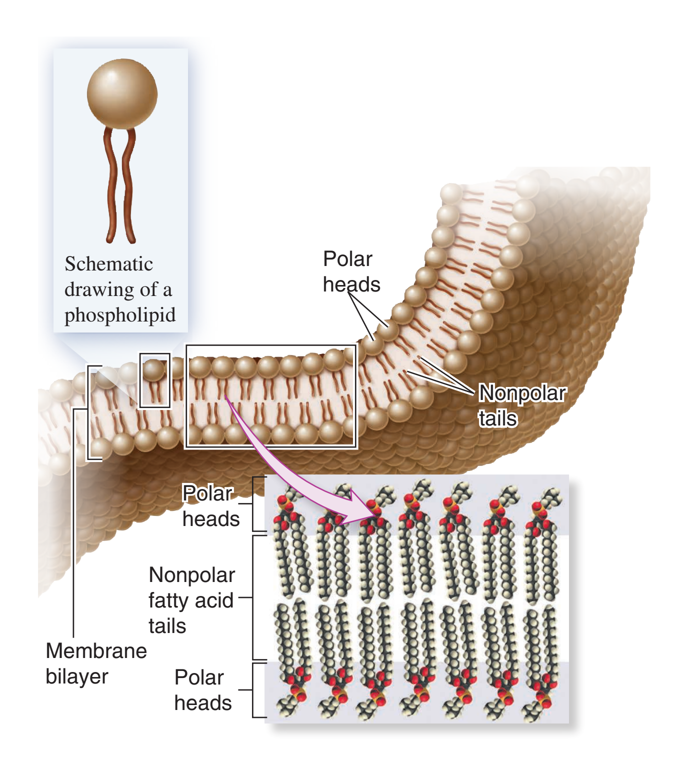


Figure 10.2 Arrangement of phospholipids in a biological membrane, such as the plasma membrane that encloses cells. The hydrophilic regions of the phospholipid face the watery environments on either side of the membrane, while the hydrophobic regions associate with each other in the interior of the membrane, thereby forming a bilayer.

The physical and chemical integrity of a membrane is crucial for the proper functioning of the cell or organelle that it surrounds. As a stable sheet of interlocking molecules, the membrane functions as a barrier to simple diffusion. In general, the permeability of a membrane to a solute depends on a combination of the solute's size, charge (ions), polarity, and lipid solubility. Small, uncharged, nonpolar, lipid-soluble molecules pass most easily through the lipid core of a membrane (fig. 10.4).

Question 1

a. What ions must routinely move across cell membranes?

b. How could membranes promote the movement of ions out of or into cells? How could membranes restrict the movement of ions out of or into cells?

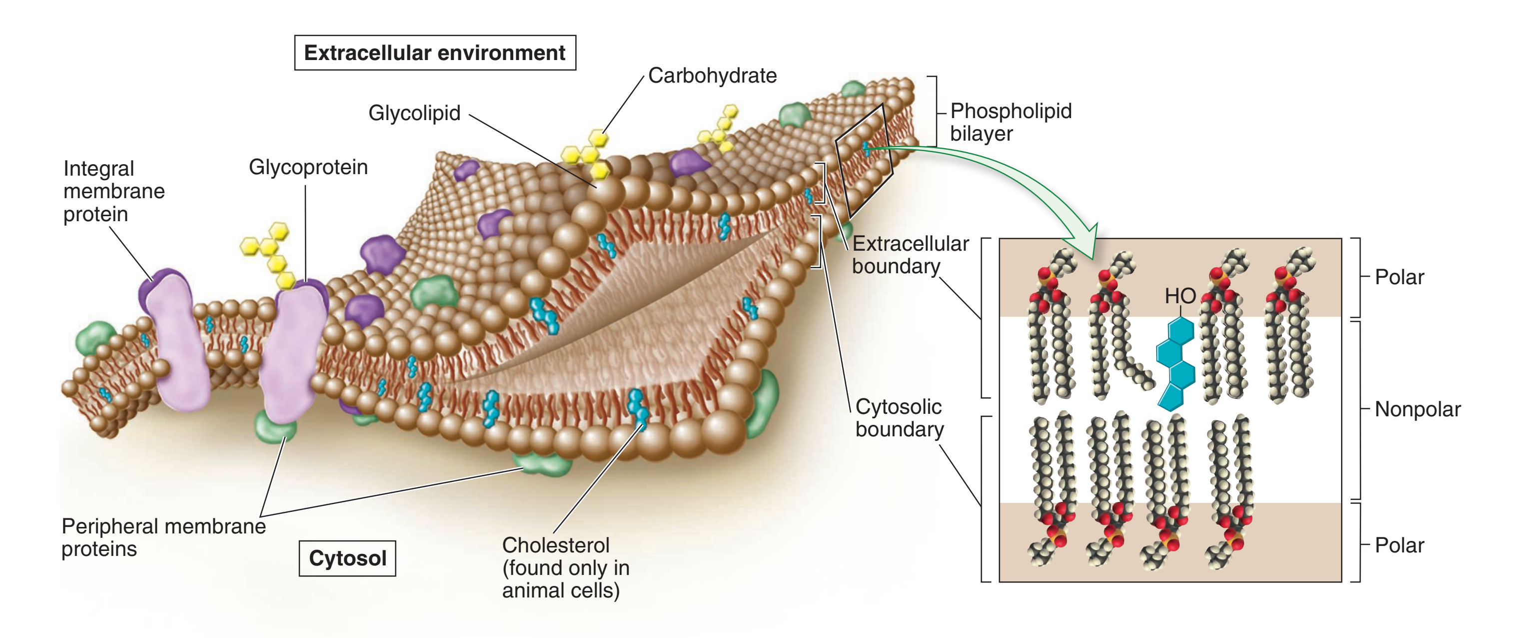


Figure 10.3 Fluid-mosaic model of membrane structure. The basic framework of a plasma membrane is a phospholipid bilayer. Proteins may span the membrane and may be bound on the surface to other proteins or lipids. Proteins and lipids, which have covalently-bound carbohydrates, are called glycoproteins and glycolipids, respectively.

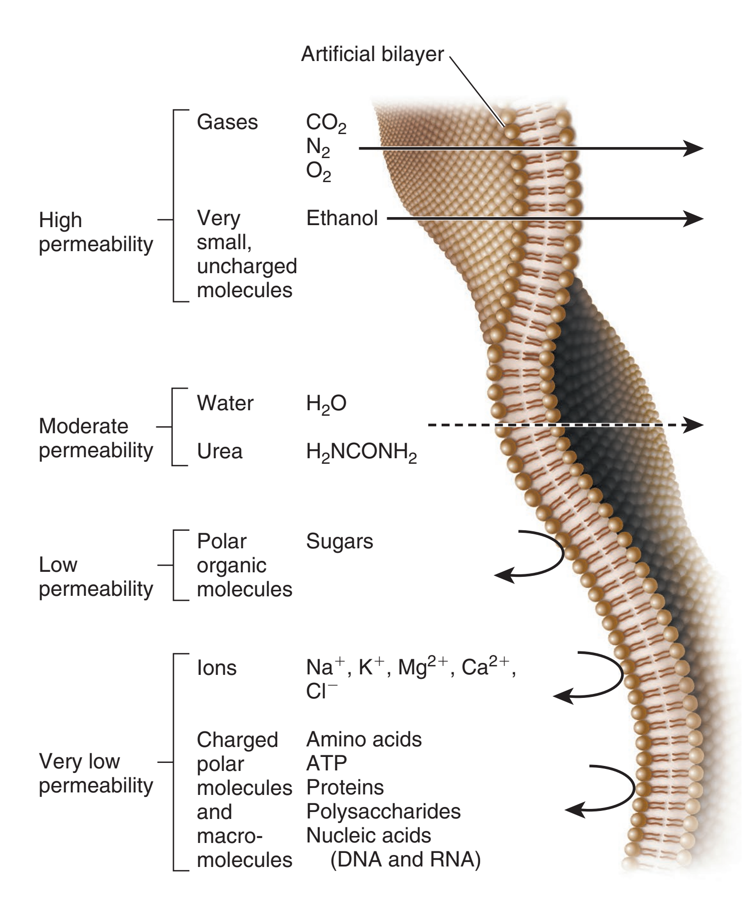


Figure 10.4 Relative permeability of an artificial phospholipid bilayer to a variety of solutes. Solutes that easily penetrate the membrane are shown with a straight arrow that passes through the bilayer. The dotted line indicates solutes that have moderate permeability. The remaining solutes shown at the bottom are relatively impermeable.

c. Could a cell survive without an intact cell membrane? Explain.

BEET CELLS AS AN EXPERIMENTAL SYSTEM

Beet tissue will be your model to investigate membrane integrity. Roots of beet (*Beta vulgaris*) contain large amounts of a reddish pigment called **betacyanin** localized almost entirely in the large central vacuoles of cells. In healthy cells, betacyanin remains inside the vacuoles, surrounded by a vacuolar membrane called the **tonoplast.** The entire cell (including the vacuole, tonoplast, and cytoplasm) is surrounded by a cell membrane and cell wall.

In two procedures you will subject beet cells to a range of temperatures and organic solvents and determine which treatments stress and damage the membranes the most. If stress damages the membranes, betacyanin will leak through the tonoplast and plasma membrane. This leakage from the stressed beet will color the surrounding water red. Thus, you can measure membrane damage by measuring the intensity of color resulting from a treatment.



SAFETY FIRST Before coming to lab you were asked to read this exercise so you would know what to do, and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

THE EFFECT OF TEMPERATURE STRESS ON MEMBRANES

Membranes are sensitive to extreme temperatures. High temperatures cause violent molecular collisions that degrade a membrane as a physical barrier to diffusion. Conversely, freezing temperatures cause water to crystallize as ice and expand because of hydrogen bond alignment. This expansion and formation of ice often rupture membranes.

Procedure 10.1 **Observe the effect of temperature stress on cellular membranes**

1. Examine the treatments listed in table 10.1. Hypothesize which treatments will cause the most and least damage. Record your rankings alongside the tube numbers in the column marked Tube Number.
2. Cut six uniform cylinders of beet using a cork borer with a 5-mm inside diameter. Trim each cylinder to exactly 15 mm in length. All of the cylinders must be the same size.
3. Place these cylinders of beet tissue in a beaker and rinse them with tap water for 2 min to wash betacyanin from the injured cells on the surface. Be sure that all of the cylinders are washed in the same way. Discard the colored rinse-water.
4. Gently place one of the six beet sections into each of six dry test tubes. Do not crush, stab, or otherwise damage the cylinders when moving them to the test tubes.
5. Label the tubes 1–6 and write the temperature treatment on each tube as listed in table 10.1.
6. **FOR COLD TREATMENTS:**
    a. Place tube 5 in a refrigerator (5°C) and tube 6 in a freezer (−5°C). If a refrigerator and freezer are not available in your lab, give your labeled tubes to an assistant who will take them to another facility.

Table 10.1

The Color Intensity of Betacyanin Leaked from Damaged Cells Treated at Six Temperatures

Tube Number	Treatment (°C)	Color Intensity (0–10)		Absorbance (460 nm)	
		Working Group	Class Average	Working Group	Class Average
1	70				
2	55				
3	40				
4	20				
5	5				
6	–5				

b. Leave tubes 5 and 6 in the cold for 30 min. While waiting, proceed with hot treatments (step 7). However, watch your time and return to steps 6c and 6d after 30 min.

c. After 30 min, remove the beets from the freezer and refrigerator and add 10.0 mL of distilled water at room temperature to each of the tubes.

d. Let the cold-treated beets soak in distilled water for 20 min. Then remove and discard the beets from tubes 5 and 6.

7. **FOR HOT TREATMENTS:**

a. Take the beet section out of tube 1 and immerse it in a beaker of hot water at 70°C for 1 min. If a 70°C water-bath is not available, hot tap water should be adequate, but carefully adjust the temperature to 70°C. Handle the beet gently with forceps, and don't squeeze it tightly because you may rupture the beet's cells.

b. After 1 min at 70°C, return the beet to tube 1 and add 10.0 mL of distilled water at room temperature.

c. If a 55°C water-bath is not available, slowly add ice chips or cold water to cool the beaker of hot water to 55°C. Then immerse the beet from tube 2 for 1 min. Return the beet to tube 2 and add 10.0 mL of distilled water at room temperature.

d. If a 40°C water-bath is not available, cool the beaker of hot water to 40°C. Then immerse the beet from tube 3 for 1 min. Return the beet to tube 3 and add 10.0 mL of distilled water at room temperature.

e. If a 20°C water-bath is not available, cool the beaker of hot water to 20°C. Then immerse the beet from tube 4 for 1 min. Return the beet to tube 4 and add 10.0 mL of distilled water at room temperature.

f. Allow the treated beets in tubes 1–4 to soak in distilled water at room temperature for 20 min. Then remove and discard the beets and measure the extent of membrane injury according to the amount of betacyanin that diffused into the water.

8. **FOR ALL SIX TEMPERATURE TREATMENTS:**
Quantify the relative color of each solution between 0 (colorless) and 10 (darkest red). If color standards are available in the lab, use them to determine relative values for the colors of your samples. Record the results for your work group in table 10.1. Also provide your results to the instructor to calculate the class averages.

Use the graph paper at the end of this exercise to graph *Temperatures* versus *Relative Color* for the class averages according to a demonstration graph provided by your instructor. Your instructor may also ask you to quantify your results further using a spectrophotometer. If so, see Exercise 8 for instructions for using a spectrophotometer. Read the absorbance of the solutions at 460 nm and record your results and the class average results in table 10.1. Then graph *Temperature* versus *Absorbance* for the class averages.

Question 2

a. Which temperature damaged membranes the most? Which the least? How do you know?

b. In general, which is more damaging to membranes, extreme heat or extreme cold? Why?

Table 10.2

The Color Intensity of Betacyanin Leaked from Damaged Cells Treated with Various Concentrations of Two Organic Solvents

Tube Number	Treatment	Color Intensity (0–10)		Absorbance (460 nm)	
		Working Group	Class Average	Working Group	Class Average
1	1% acetone				
2	25% acetone				
3	50% acetone				
4	1% methanol				
5	25% methanol				
6	50% methanol				
7	Isotonic saline				

c. If the results of this experiment are easily observed with the unaided eye, why use a spectrophotometer?

d. The beets were subjected to cold temperatures longer than to hot temperatures to make sure that the beet sections were thoroughly treated. Why does the freezing treatment require more time?

e. How accurate were your hypothesized rankings for the organic solvent treatments?

THE EFFECT OF ORGANIC SOLVENT STRESS ON MEMBRANES

Organic solvents dissolve a membrane's lipids. Acetone and methanol are common solvents for various organic molecules, but acetone has the greater ability to dissolve lipids.



Organic solvents are flammable. Extinguish all open flames and heating elements before doing the following procedure. Do not pour organic solvents down the drain. Dispose of them properly. Use a fume hood as directed by your instructor.

Question 3
Which of the organic liquids (acetone or methanol) do you predict will damage membranes the most?

Procedure 10.2 **Observe the effect of organic solvents on cellular membranes**

1. Examine the treatments listed in table 10.2. Hypothesize which treatments will cause the most and least damage. Note your rankings alongside the tube numbers in the column marked Tube Number.
2. Cut seven uniform cylinders of beet using a cork borer with a 5-mm inside diameter. Trim each cylinder to exactly 15 mm in length. All the cylinders must be the same size.
3. Place these cylinders of beet tissue in a beaker and rinse them with tap water for 2 min to wash betacyanin from the injured cells on the surface. Be sure that all of the cylinders are the same size. Discard the colored rinse-water.
4. Place one of the seven beet sections into each of seven dry test tubes. Do not crush, stab, or otherwise damage the cylinders when moving them to the test tubes.
5. Label the tubes 1–7 and write the organic-solvent treatment on each tube as listed in table 10.2.
6. Add 10.0 mL of the appropriate solvent (see table 10.2) to each of the seven tubes.
7. Keep all beets at room temperature for 20 min and shake them occasionally. Then remove and discard the beet sections and measure the extent of membrane

damage according to the amount of betacyanin that diffused into the water.

8. Quantify the relative color of each solution between 0 (colorless) and 10 (darkest red). If color standards are available in the lab, use them to determine relative values for the colors of your samples. Record the results for your work group in table 10.2. Also provide your results to the instructor to calculate the class averages.



Be sure to dispose of the organic solvents as directed by your instructor.

Graph *Concentration of Organic Solvent* versus *Relative Color* for the class averages according to a demonstration graph provided by your instructor. Your instructor may also ask you to further quantify your results using a spectrophotometer. If so, see Exercise 8 for instructions for using a spectrophotometer. Read the absorbance of the solutions at 460 nm and record your results and the class average results in table 10.2. Then graph *Concentration of Organic Solvent* versus *Absorbance* for the class averages.

Question 4

a. Based on your results, are lipids soluble in both acetone and methanol?

b. Based on your results, which damages membranes more: 50% methanol or 25% acetone?

c. In which solvent are lipids most soluble?

d. The concentration of solvent affects its ability to dissolve lipids. Based on your results, did the highest concentration of both solvents cause the most damage?

e. What other solvents might be interesting to test in this experiment?

f. What was the purpose of tube 7?

g. How accurate were your hypothesized rankings for the temperature treatments?

INVESTIGATION

Effects of Environmental Stimuli on Cellular Membranes

Observation: Cellular membranes are the interface between cells and their environment. The integrity of cellular membranes, which is critical for the proper functioning of the membranes and cells, is affected by environmental stimuli.

Question: How do solvents or temperature affect membrane permeability?

a. Establish a working lab group and obtain Investigation Worksheet 10 from your instructor.
b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.
c. Translate your question into a testable hypothesis and record it.
d. Outline on Worksheet 10 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.
e. Conduct your procedures, record your data, answer your question, and make relevant comments.
f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

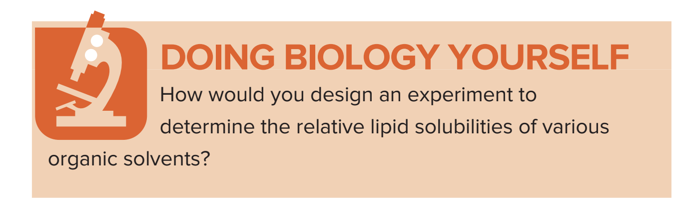
1. Are your conclusions about membrane structure and stress valid only for beet cells? Why or why not?

2. What characteristics of beets make them useful as experimental models for studying cellular membranes?

3. Explain why phospholipids have a natural tendency to self-assemble into a bilayer. Why is this biologically important?

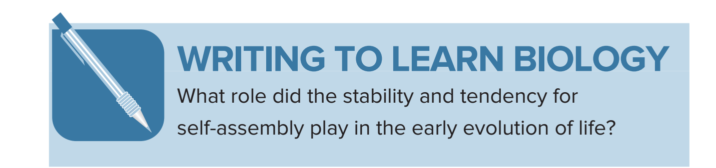
4. Freezing temperatures are often used to preserve food. Considering the results of this experiment, which qualities of food are preserved and which are not?

5. Movement of water through membranes has long puzzled scientists. Why would you not expect water to move easily through a membrane?



DOING BIOLOGY YOURSELF

How would you design an experiment to determine the relative lipid solubilities of various organic solvents?



WRITING TO LEARN BIOLOGY

What role did the stability and tendency for self-assembly play in the early evolution of life?

Enzymes

Factors Affecting the Rate of Activity

Learning Objectives

By the end of this exercise you should be able to:

1. Describe the relationship between structure and function of enzymes.
2. Relate structure and function to active sites, modes of inhibition, and optimal conditions for enzymatic activity.
3. Hypothesize and test how inhibitors and changes in temperature and pH affect enzymatic reaction rates.
4. Describe how some enzymatic reaction rates can be measured by color changes and gas liberation as products are formed.

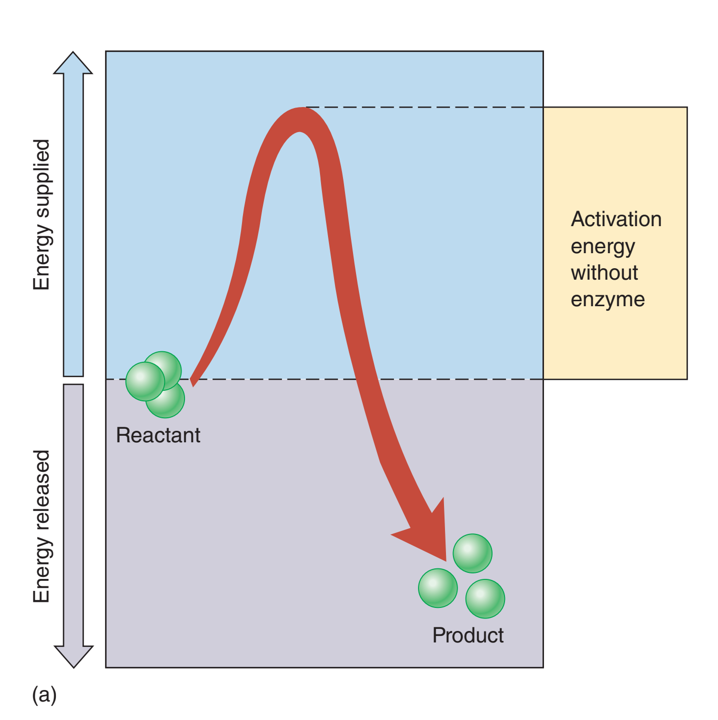


Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Fortunately, not all chemical reactions within our cells occur spontaneously. If they did, our metabolism would be chaotic. Instead, most reactions in cells are controlled by proteins called **enzymes.** Enzymes are **biocatalysts,** meaning that they accelerate metabolic reactions to biologically useful rates. Specifically, enzymes catalyze (accelerate) reactions by lowering the activation energy needed for the reaction to occur (fig. 11.1).

Enzymes bind to reacting molecules called the **substrate,** to form an **enzyme-substrate** complex. This complex stresses or distorts chemical bonds and forms a **transition state** in which the substrate becomes more reactive. The energy needed to form the transition state is called **energy of activation** and is lowered by the enzyme. The site of attachment and the surrounding parts of the enzyme that stress the substrate's bonds constitute the enzyme's **active site** (fig. 11.2).

The reaction is complete when the **product** forms and the enzyme is released in its original condition. The enzyme



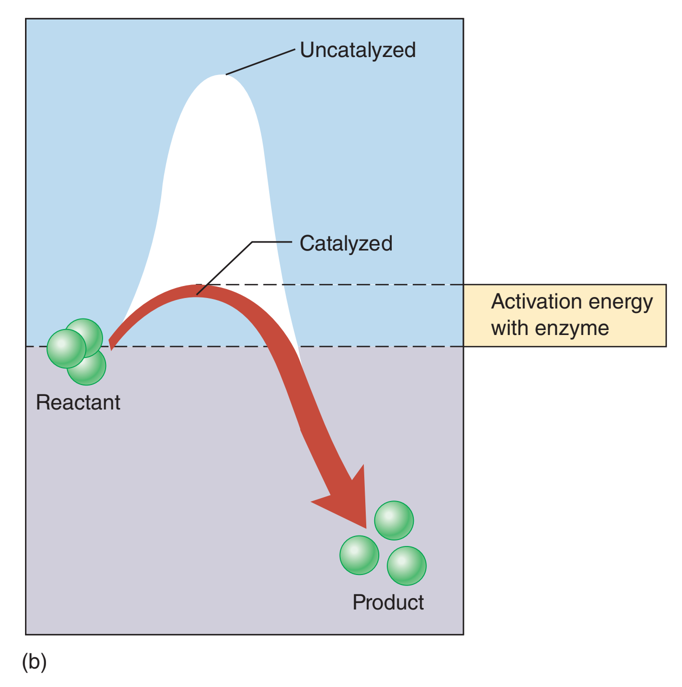


Figure 11.1 Activation energy and catalysis. (*a*) Exergonic reactions (those that release energy) do not necessarily proceed rapidly because energy must be supplied to destabilize existing chemical bonds. This extra energy is the activation energy for the reaction. (*b*) Catalysts such as enzymes accelerate particular reactions by lowering the amount of activation energy required to initiate the reaction.

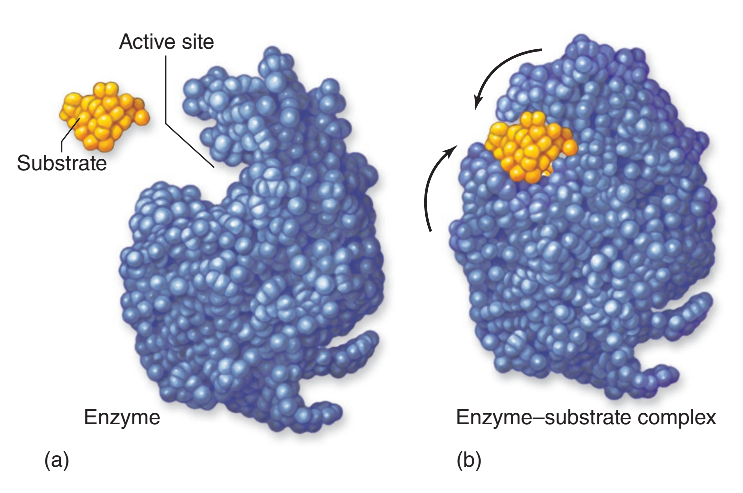


Figure 11.2 Enzyme binding its substrate. (*a*) The active site of an enzyme fits the shape of its substrate. (*b*) When the substrate, indicated in yellow, slides into the active site, the protein binds the substrate tightly and slightly alters the substrate's shape.

then repeats the process with other molecules of substrate (fig. 11.3). Enzymes are reusable.

Enzyme + substrate ⟶ enzyme-substrate complex ⟶ enzyme + product

Enzymes are proteins made of long chains of amino acids that form complex shapes. Although cells contain many enzymes, each enzyme has a precise structure and function, and catalyzes a specific reaction. This specificity results from an enzyme's unique structure and shape. The complex shape of the active site on the enzyme's surface usually couples with only one type of substrate.

Any structural change in an enzyme may **denature** or destroy its effectiveness by altering the active site and slowing the reaction. Denatured enzymes may result from extreme temperature, extreme pH, or any environmental condition that fundamentally alters a protein's structure. Therefore, the rate of an enzymatic reaction depends on conditions in the immediate environment. These conditions affect the shape of the enzyme and modify the active site and precise fit of an enzyme and its substrate.

The range of values for environmental factors such as temperature and pH within which an enzyme functions best represents that enzyme's **optimal conditions.** The optimal conditions for the enzymes of an organism are usually adaptive for the environment of the organism. Other factors such as the amount of substrate or concentration of enzyme also affect the reaction rate.

In this exercise you will learn that environmental factors such as temperature and pH affect enzymatic reactions (fig. 11.4). You will also investigate how inhibitors affect enzymatic activity.

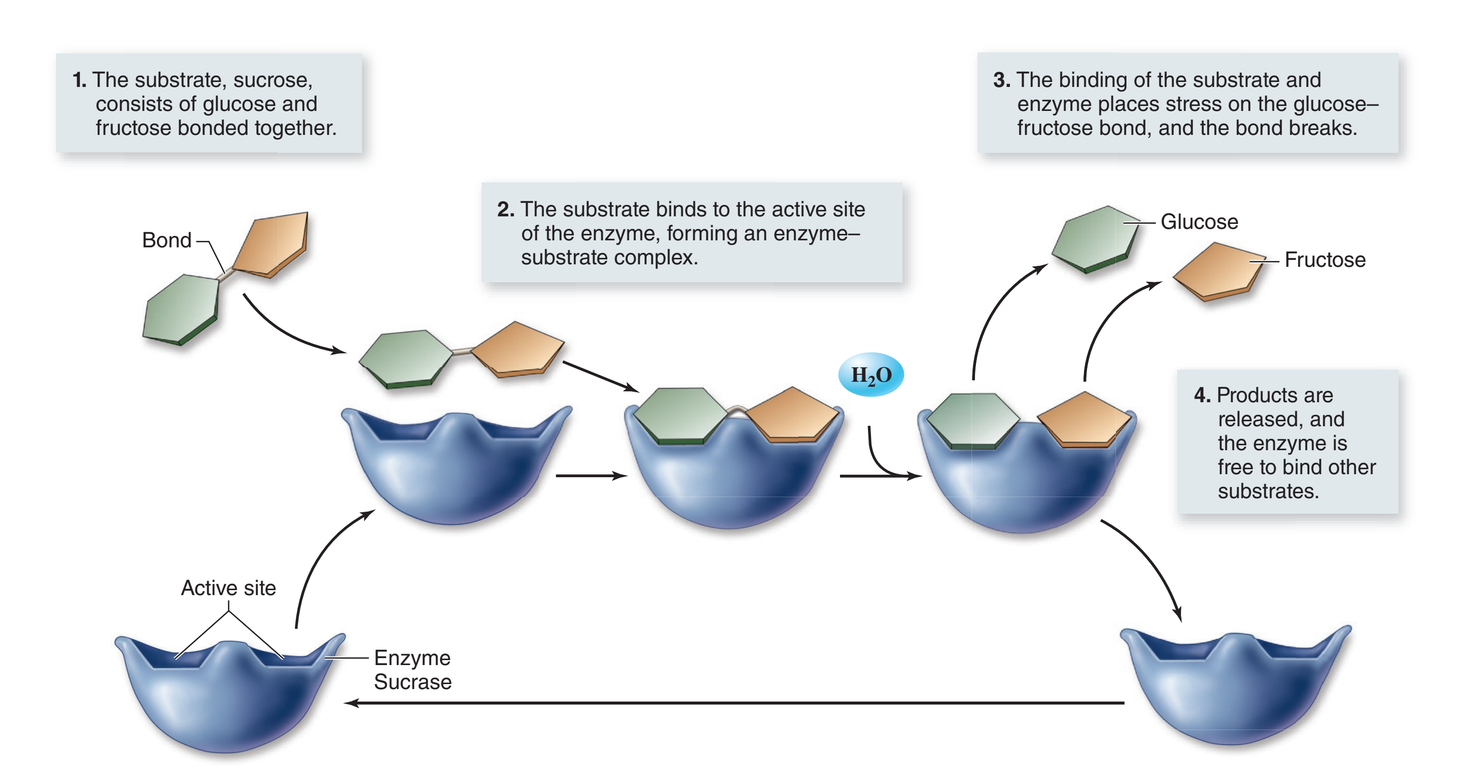
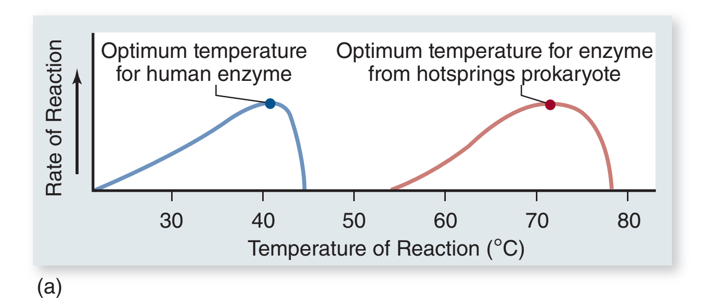
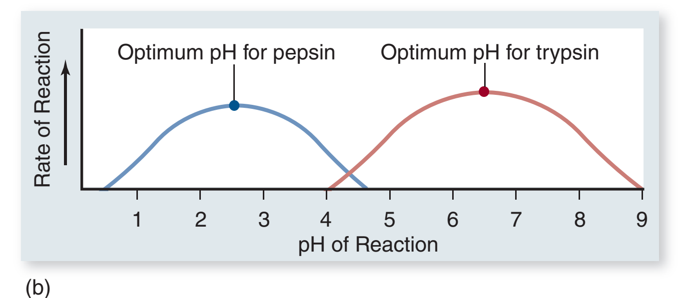


Figure 11.3 The catalytic cycle of an enzyme. Enzymes increase the speed of chemical reactions but are not themselves permanently altered by the process. Here, the enzyme sucrase splits the disaccharide sucrose (steps 1, 2, 3, and 4) into its two parts, the monosaccharides glucose and fructose. After the enzyme releases the glucose and fructose, it can bind another molecule of sucrose and begin the catalytic cycle again.



(a)



(b)

Figure 11.4 Enzymes are sensitive to their environment. The activity of an enzyme is influenced by both (*a*) temperature and (*b*) pH. Most enzymes in humans, such as the protein-degrading enzyme trypsin, work best at temperatures about 40°C and within a pH range of 6 to 8. As you can see, however, pepsin works best at a much lower pH than does trypsin.

TEMPERATURE AFFECTS THE ACTIVITY OF ENZYMES

Heat increases the rate of most chemical reactions. During enzymatic reactions, faster molecular motion caused by heat increases the probability that enzyme molecules will contact substrate molecules. The rate of chemical reactions generally doubles with a 10°C rise in temperature. However, higher temperatures do not always accelerate enzymatic reactions; enzymatic reactions have an optimal range of temperatures. Temperatures above or below this range decrease the reaction rate. Extreme temperatures often denature enzymes.

The effects of temperature on enzyme activity can be investigated with **catechol oxidase,** a plant enzyme that converts catechol to benzoquinone. When fruit is bruised, injured cells release catechol and catechol oxidase, which react to form a brownish product, benzoquinone. Toxic to bacteria, benzoquinone prevents decay in damaged cells. Your source of catechol oxidase will be potato extract.

$$\underset{\textbf{(substrate)}}{\textbf{Catechol}} \xrightarrow[\textbf{(enzyme)}]{\textit{catechol oxidase}} \underset{\textbf{(product)}}{\textbf{benzoquinone}}$$



Catechol is toxic. Wash well with soap and water after skin contact.



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 11.1 Determine the effect of temperature on catechol oxidase activity

1. Read all steps in this procedure and state the null hypothesis that your experiments will test.
2. Prepare water-baths at 40°C and 80°C. Locate a refrigerator or ice bath at or below 4°C. Place a test-tube rack in each bath and in the refrigerator.
3. Obtain seven test tubes and number them at the top 1–7.
4. Obtain a tube of potato extract and a tube of 1% catechol from your instructor.
5. Add distilled water, pH buffer, and potato extract to the tubes as listed in table 11.1. Shake or swirl to resuspend the potato extract.
6. Place the tubes in the appropriate bath or refrigerator. Allow each tube to stand undisturbed for 5 min at its respective temperature. Put tubes 1–4 in a test-tube rack at room temperature (approximately 22°C).
7. Add 1% catechol solution to tubes 2 and 4–7 as listed in table 11.1. For each tube immediately record in table 11.2 any color changes for 0 min. Record qualitative color changes on a scale between 0 (no change) and 5 (drastic change).
8. Every 5 min observe and note color changes in the seven tubes over the next 20 min. Always return the tubes to their original temperature locations (e.g., refrigerator, water-bath).
9. If your instructor asks you to further quantify your data, then measure the absorbance of the solution in each tube using a spectrophotometer set to 470 nm with tube 3 as a blank. Refer to Exercise 8 and the videos tailored to that exercise for instructions on how to use the spectrophotometer.
10. Clean your work area and materials. **Catechol must be disposed into waste containers, not down the sink drain.**

Question 1

a. Do your data support or refute your hypotheses?

Table 11.1

Experimental Conditions to Test the Effect of Temperature on Catechol Oxidase Activity

Tube	Distilled Water	pH 6 Buffer	Potato Extract (catechol oxidase)	1% Catechol	Temperature
1	2 mL	1 mL			22°C
2	1 mL	1 mL		1 mL	22°C
3	1 mL	1 mL	1 mL		22°C
4		1 mL	1 mL	1 mL	22°C
5		1 mL	1 mL	1 mL	4°C
6		1 mL	1 mL	1 mL	40°C
7		1 mL	1 mL	1 mL	80°C

Table 11.2

Qualitative and Quantitative Color Changes as Catechol Oxidase Activity Produces Brown Benzoquinone

	Qualitative Color Change Results						Quantitative Absorbance Results				
Tube	0 min	5 min	10 min	15 min	20 min		0 min	5 min	10 min	15 min	20 min
1	0	0	0	0	0		0	0	0	0	
2											
3											
4											
5											
6											
7											

b. Write a hypothesis and a null hypothesis for the effect of temperature on catechol oxidase activity.

c. What were the enzyme, substrate, and product of the enzymatic reaction?

d. Why was each tube left undisturbed for 5 min in step 6 of procedure 11.1?

e. Explain the results observed for tubes 1–3. What was the purpose of these tubes?

f. Use your results for tubes 4–7 to construct a line graph of *Enzyme Activity* versus *Time* on graph paper provided at the end of the exercise. Your graph will include four curves.

g. Use your results to argue for or against the statement, "Catechol oxidase functions equally and efficiently at various temperatures."

h. Over what range of temperatures tested was catechol oxidase active? Should other temperatures be tested to more accurately determine the range of activity?

i. At which temperature was catechol oxidase activity greatest? Should more temperatures be tested to determine its optimum?

j. At what temperature was catechol oxidase denatured? How do you know?

k. What is the effect of denaturing an enzyme?

l. If an enzyme has a single optimal temperature, then an organism might have difficulty dealing with an environment with wide temperature variation. What adaptive advantage is there in having repetitive enzyme systems (i.e., more than one enzyme to catalyze the same reaction) that we know many organisms have?

pH AFFECTS THE ACTIVITY OF ENZYMES

Enzymatic activity is sensitive to pH. Acidic and basic solutions are rich in H^+ and OH^- ions (see Exercise 5), respectively, and they readily react with the charged side groups of the enzyme molecules. As the pH is lowered, side groups gain H^+ ions; as the pH is raised, side groups lose H^+ ions. In this way, solutions having an extreme pH can change an enzyme's shape enough to alter its active site. Extreme pH can denature an enzyme just as drastically as can high temperatures. Many

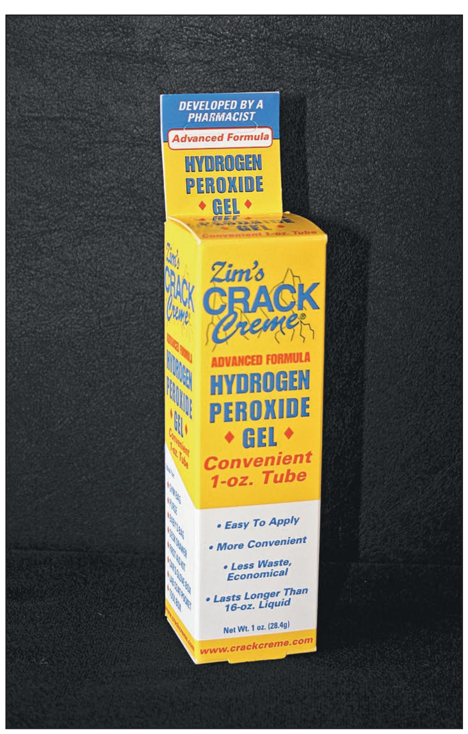


Figure 11.5 Ointments with high concentrations of hydrogen peroxide can be effective antibiotics. Hydrogen peroxide is a naturally produced toxin in cells and is degraded by the enzyme catalase.

enzymes function optimally in the neutral pH range, while others (such as pepsin, an enzyme in your digestive tract) function best at pH as low as 1.6 (fig. 11.4*b*).

The effects of pH can be investigated with **catalase,** an enzyme in plants and animals that speeds the breakdown of hydrogen peroxide, toxic to cells. It may surprise you that cells can produce a toxin naturally. Could that ability be useful to cells? Occasionally we take advantage of hydrogen peroxide as a powerful oxidizer that denatures macromolecules and kills "germs" (fig. 11.5). Hydrogen peroxide is broken down by catalase to water and oxygen.

$$2\,H_2O_2 \xrightarrow{\textit{catalase}} 2\,H_2O + O_2$$

Procedure 11.2 **Observe the effects of pH on catalase activity**

1. Read all steps in this procedure and state the null hypothesis that your experiments will test.
2. Prepare catalase solution.
   - *a.* Use a mortar and pestle to macerate a marble-size portion of fresh, raw ground meat in 10 mL of distilled water.
   - *b.* Filter the solution through cheesecloth into a test tube and add an equal volume of distilled water.
3. Obtain 10 test tubes and number them at the top 1–10.

Table 11.3

Experimental Conditions to Test the Effect of pH on Catalase Activity

Tube	Distilled Water	Buffer	Hydrogen Peroxide	HCl	NaOH	pH	Catalase Solution
1	5 mL	1 mL, pH 7					
2	4 mL	1 mL, pH 7					1 mL
3	2 mL	1 mL, pH 7	3 mL				
4	1 mL		3 mL	1 mL			1 mL
5	1 mL	1 mL, pH 5	3 mL				1 mL
6	1 mL	1 mL, pH 7	3 mL				1 mL
7	1 mL	1 mL, pH 9	3 mL				1 mL
8	1 mL		3 mL		1 mL		1 mL
9	1 mL	1 mL, pH 7	3 mL	1 mL			
10	1 mL	1 mL, pH 7	3 mL		1 mL		

4. Obtain stock solutions of distilled water, hydrogen peroxide, buffer pH 5, buffer pH 7, buffer pH 9, 0.1 M HCl, and 0.1 M NaOH.



HCl is a strong caustic acid, and NaOH is a strong caustic base. Follow your instructor's directions for handling, dispensing, and disposing of these chemicals. Rinse immediately with water if you spill any acid or base on your skin.

5. Add distilled water and hydrogen peroxide to each tube as listed in table 11.3. If you are measuring by drops, then 1 mL equals about 20 medium-sized drops. Wait 2 min before proceeding to step 6.

6. Add 1 mL of HCl to tubes 4 and 9. Verify that the pH is approximately 3 or lower.

7. Add 1 mL of NaOH to tubes 8 and 10. Verify that the pH is approximately 11 or higher.

8. Add 1 mL of the buffer solutions as indicated in table 11.3.

9. Your instructor may ask you to verify that the buffers produce the indicated pH. If so, use pH paper to measure the values for each solution and record them in table 11.3.

10. No catalase is added to tubes 1, 3, 9, or 10.

11. Add catalase to tube 2 according to table 11.3. After adding catalase, swirl the solution gently and immediately record in table 11.4 qualitative changes in the bubbling intensity of oxygen production on a scale of 0 (no bubbling) to 5 (vigorous bubbling).

12. If your instructor asks you to more rigorously quantify your results, then immediately after adding the catalase place a stopper with tubing over each tube to collect and measure the volume of gases produced in a water-filled graduated cylinder inverted in a beaker of water (fig. 11.6). Be sure that the graduated cylinder does not pinch off the rubber tubing. Also be sure the cylinder is vertical when you measure volume. Record these results in table 11.4.

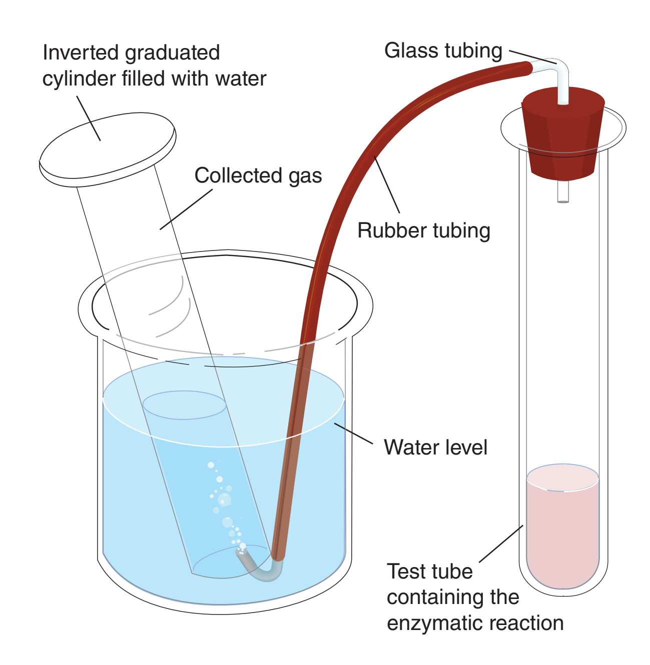


Figure 11.6 A method to capture oxygen released by catalase activity.

13. Repeat step 11 for each remaining solution.

14. After you have gathered your data for all 10 tubes, record in table 11.4 your explanation for the results of the catalase activity in each of the tubes.

15. Clean your work area and materials. Follow your instructor's directions concerning the disposal of waste solutions containing HCl and NaOH.

Table 11.4

Production of Oxygen by Catalase Activity. Qualitative Data Are Observations of Intensity of Oxygen Effervescence Ranging from 1–5. Quantitative Data Are Milliliters of Oxygen Produced.

Tube	Oxygen Production		Explanation
	Qualitative (0–5)	Quantitative (mL O_2)	
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Question 2

a. Do your data support or refute your hypothesis?

b. What were the enzyme, substrate, and product of the enzymatic reaction?

c. What was the purpose of completing steps 2–9 for all tubes before adding the catalase in step 11?

d. What was the purpose of tubes 1, 2, 3, 9, and 10?

e. Use your data for tubes 4–8 to construct a line graph of *Enzyme Activity* versus *pH.*

f. Over what pH range was catalase active?

g. What pH levels denatured catalase? Specifically how do solutions of high or low pH change an enzyme's reactivity?

h. At which of the tested pH values did catalase react most rapidly? Should more values be tested to accurately determine its optimum?

i. After experimenting with the effects of pH on enzymes, would you suspect that human blood has a constant pH? Why? What would be the adaptive advantage of this?

INHIBITORS AFFECT THE ACTIVITY OF ENZYMES

Peroxidase is an enzyme in plants (such as turnips) and some bacteria that converts toxic hydrogen peroxide to H_2O and O_2 in a reaction similar to that of catalase. Peroxidase is a large protein with a reactive iron atom at its active site.

$$2\ H_2O_2 \xrightarrow{\textit{peroxidase}} 2\ H_2O + O_2$$

Enzymes such as peroxidase can be inhibited by chemicals in various ways. One mechanism is **competitive inhibition.** Competitive inhibitors are molecules structurally similar to the substrate and therefore competitive for positions at the active sites of enzymes. This ties up the enzyme and makes it unable to bind with the substrate. For example, hydroxylamine ($HONH_2$) is structurally similar to hydrogen peroxide (H_2O_2) and binds to the iron atom at the active site of peroxidase. Thus, hydroxylamine competes with hydrogen peroxide for the active sites on peroxidase molecules and reduces the frequency of hydrogen peroxide binding with peroxidase molecules. However, a high enough concentration of enzyme with a constant concentration of inhibitor can reduce the inhibition.

The production of oxygen by peroxidase provides a method to measure the ongoing reaction rate. One method would be to capture liberated bubbles of oxygen and measure their total volume. But in the following procedure you will measure oxygen by combining it with a dye that changes color when it is oxidized. Guaiacol is a convenient dye that turns from colorless to brown as it is oxidized by oxygen. The amount of brown color in the final product is proportional to the amount of oxygen formed by the reaction. You can measure the color change qualitatively by rating the color of a reacting solution on an arbitrary scale from 0 to 5, or quantitatively with a spectrophotometer measuring the solution's absorbance of 470 nm light. Review Exercise 8 and the associated video for instructions on using a spectrophotometer.

$$\underset{\text{(colorless)}}{O_2 + \text{guaiacol}} \longrightarrow \underset{\text{(brown)}}{\text{oxidized guaiacol}}$$

Procedure 11.3 Observe the effects of an inhibitor on enzymatic activity

1. Read all steps in this procedure and state the null hypothesis that your experiments will test.
2. Prepare turnip extract.
   - *a.* Thoroughly blend 6 g of the inner portion of a peeled turnip in a blender with 200 mL of cold water.
   - *b.* Filter the turnip slurry through cheesecloth into a beaker.
   - *c.* Pour about 7 mL of the extract into a test tube and determine its absorbance in a spectrophotometer. Refer to Exercise 8 for instructions on spectrophotometry. The absorbance for the turnip extract should be between 0.1 and 0.2 at 470 nm to give a reasonable concentration of enzyme.
   - *d.* Dilute or concentrate the suspension as necessary. Your instructor may provide directions for standardizing this enzyme solution more precisely.
3. Obtain nine test tubes and number them at the top 1–9.
4. Add distilled water to each tube as listed in table 11.5.
5. To tube 1, add 0.1 mL (2 drops) of guaiacol as listed in table 11.5 and swirl the contents.
6. Immediately determine the solution's absorbance at 470 nm using a spectrophotometer. Record the absorbance value in table 11.6. Measure the absorbance every 30 sec for 5 min and record the value each time.
7. To tube 2, add 0.2 mL (4 drops) of hydrogen peroxide as listed in table 11.5. Swirl the contents. Repeat step 6 quickly.
8. To tube 3, add 0.1 mL (2 drops) of guaiacol and 0.2 mL of hydrogen peroxide as listed in table 11.5. Swirl the contents. Repeat step 6 quickly.
9. To tube 4, add 0.1 mL (2 drops) of guaiacol and 1.0 mL of turnip extract as listed in table 11.5. Swirl the contents. Repeat step 6 quickly.
10. Complete all of the measurements for steps 5–9 before proceeding to step 11.
11. For each of tubes 5–9, add 0.1 mL (2 drops) of guaiacol and 0.2 mL (4 drops) of hydrogen peroxide as listed in table 11.5.
12. For tube 5, add 1.0 mL of turnip extract and swirl the contents. Repeat step 6 quickly.
13. For tube 6, add 1.0 mL of turnip extract and 0.5 mL (10 drops) of hydroxylamine and swirl the contents. Repeat step 6 quickly.
14. For tube 7, add 1.5 mL of turnip extract and 0.5 mL (10 drops) of hydroxylamine and swirl the contents. Repeat step 6 quickly.
15. For tube 8, add 2.0 mL of turnip extract and 0.5 mL (10 drops) of hydroxylamine and swirl the contents. Repeat step 6 quickly.
16. For tube 9, add 3.0 mL of turnip extract and 0.5 mL (10 drops) of hydroxylamine and swirl the contents. Repeat step 6 quickly.
17. Clean your work area and materials.

Question 3

a. Do your data support or refute your hypothesis?

b. What were the enzyme, substrate, and product of this enzymatic reaction?

Table 11.5

Experimental Conditions to Test the Inhibition of Hydroxylamine on Peroxidase Activity

Tube	Distilled Water	Guaiacol (25 mM)	Hydrogen Peroxide (3%)	Turnip Extract	Hydroxylamine (10%)
1	5.9 mL	0.1 mL			
2	5.8 mL		0.2 mL		
3	5.7 mL	0.1 mL	0.2 mL		
4	4.9 mL	0.1 mL		1.0 mL	
5	4.7 mL	0.1 mL	0.2 mL	1.0 mL	
6	4.2 mL	0.1 mL	0.2 mL	1.0 mL	0.5 mL
7	3.7 mL	0.1 mL	0.2 mL	1.5 mL	0.5 mL
8	3.2 mL	0.1 mL	0.2 mL	2.0 mL	0.5 mL
9	2.2 mL	0.1 mL	0.2 mL	3.0 mL	0.5 mL

Table 11.6

Absorbance at 470 nm of Peroxide/Peroxidase Solutions

Tube	0.0 min	0.5 min	1.0 min	1.5 min	2.0 min	2.5 min	3.0 min	3.5 min	4.0 min	4.5 min	5.0 min
1											
2											
3											
4											
5											
6											
7											
8											
9											

c. Explain the results you observed for tubes 1, 2, 3, and 4. What was the purpose of these tubes?

d. Use your data for tubes 5–9 to construct a line graph of *Enzyme Activity (Absorbance)* versus *Time.* There will be five curves on the graph. You will not graph the values for tubes 1–4.

e. In which tubes was peroxidase still active after 5 min?

f. How does hydroxylamine affect peroxidase activity?

g. Was it possible to detect peroxidase activity in the presence of the inhibitor by increasing enzyme concentration? Why or why not?

h. Inhibitors are common in biological systems. Why might some organisms release enzyme inhibitors into their surrounding environment?

INVESTIGATION

Factors Affecting the Rate of Enzymatic Activity

Observation: Numerous factors affect enzyme reaction rates. Some products inhibit activity, whereas others can stimulate activity. You learned in an earlier lab that acids, bases, and buffers affect pH, and you learned in this lab that pH affects enzymatic activity.

Question: How do common antacids affect enzymatic activity?

a. Establish a working lab group and obtain Investigation Worksheet 11 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 11 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. More substrate increases the probability that an enzyme will contact substrate and should increase the enzymatic reaction rate. How do you explain the increase in time to complete hydrolysis when more substrate was present?

2. What term describes the alteration of an enzyme's structure? What factors in addition to temperature influence a protein's structure?

3. What happens when an enzyme is denatured?

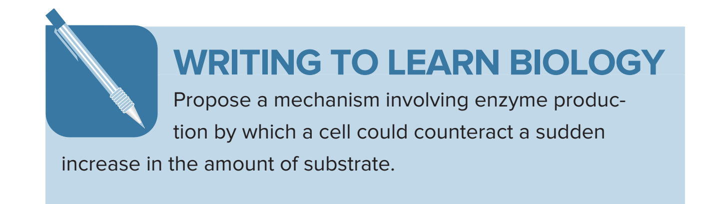
4. Can a denatured enzyme be "re-natured"? Explain your answer.

5. Commercial meat tenderizers contain papain (extracted from papaya) and/or bromelain (extracted from pineapple), both of which are enzymes. Because these enzymes "tenderize" meat, what group of organic compounds that you studied in Exercise 6 do you suspect that these enzymes react with? How could you test your answer?

6. Enzymes are proteins, and therefore are structurally linked to DNA sequences. How could natural selection alter the metabolism of an organism?

DOING BIOLOGY YOURSELF

Review the structure of starch and the action of the enzyme amylase. Design an experiment that uses a spectrophotometer to detect the progress and completion of hydrolysis of starch by amylase.



WRITING TO LEARN BIOLOGY

Propose a mechanism involving enzyme production by which a cell could counteract a sudden increase in the amount of substrate.

Respiration

Aerobic and Anaerobic Oxidation of Organic Molecules

Learning Objectives

By the end of this exercise you should be able to:

1. Demonstrate carbon dioxide production during anaerobic respiration.
2. Understand the effects of inhibitors, intermediate compounds, and cofactors in anaerobic respiration.
3. Determine oxygen consumption during aerobic respiration.
4. Use a pH-indicator to measure the relative production of carbon dioxide by plants and animals.
5. Use a respirometer to determine the metabolic rate of an animal.
6. Demonstrate practical applications of anaerobic respiration, such as making wine and kimchee.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

All living organisms respire, meaning that they have metabolic pathways that release energy from organic (rarely inorganic) molecules and capture it in ATP. Some need oxygen to do it, some don't, but they all respire because all organisms need usable chemical energy to fuel their life processes. Respiration is the chemistry that provides that energy. Usually, organic carbon molecules are the energy source, and CO_2 and H_2O are released as waste. Humans release the waste as they exhale. Respiring yeast don't exhale, but they can "pump up" rising bread by liberating CO_2 as the yeast breaks down sugar during respiration (fig. 12.1).

Cellular respiration involves oxidation of organic molecules and a concomitant release of energy. Some of this energy is stored in chemical bonds of **adenosine triphosphate (ATP),** which is used later as a direct source of energy for cellular metabolism. Organisms use the energy stored in ATP to do work such as transport materials, synthesize new compounds, reproduce, contract muscles, and remove wastes.

Photosynthesis, the topic of Exercise 13, uses light energy to split H_2O and harvest high-energy electrons. These energetic electrons (and accompanying H^+) are passed to CO_2, thereby reducing CO_2 to energy-storing sugars. Respiration removes electrons from (i.e., oxidizes) glucose, captures some of the energy in ATP, and ultimately passes the electrons to oxygen to form H_2O.



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Figure 12.1 Bread dough rises because respiring yeasts break down sugars to obtain their energy for growth and liberate CO_2, thereby forming small bubbles that cause the dough to rise. The lower loaf has been rising 4 hours longer than the upper loaf.

In most cells, respiration begins with the oxidation of glucose to pyruvate via a set of chemical reactions called **glycolysis** (fig. 12.2*a*). During glycolysis, some of the energy released from each glucose molecule is stored in ATP. Glycolysis occurs with or without oxygen. If oxygen is present, most organisms continue respiration by oxidizing pyruvate to CO_2 via chemical reactions of the **Krebs cycle.** Organisms that use oxygen for respiration beyond glycolysis are called **aerobes.**

As aerobes oxidize the acetyl group from the pyruvate in the Krebs cycle, they store energy in electron carriers such as NAD^+ (nicotinamide adenine dinucleotide). Specifically, aerobes store energy by reducing (adding high-energy electrons to) NAD^+ and FAD^+. These compounds later transfer their high-energy electrons to a series of compounds collectively called the electron transport chain. The **electron transport chain** generates proton gradients from energy stored in reduced NAD and related compounds that lead to

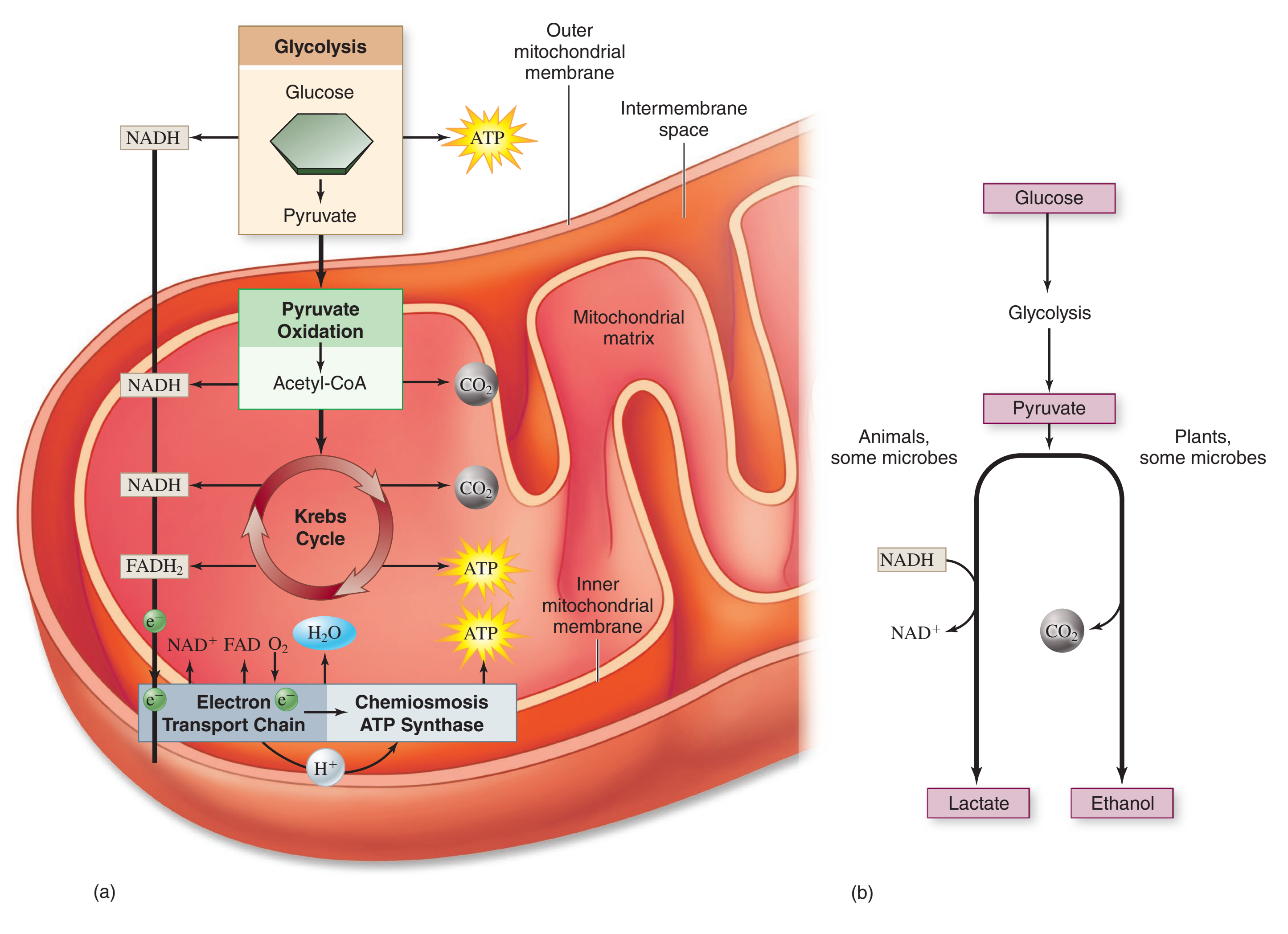


Figure 12.2 (*a*) An overview of aerobic respiration. Glycolysis occurs in the cytoplasm, and the Krebs cycle and electron transport chain occur in mitochondria. (*b*) During anaerobic fermentation, pyruvate is reduced with electrons extracted during glycolysis and carried by NADH. In organisms that reduce pyruvate directly, as in muscle cells, the product is lactate. In organisms that first remove carbon dioxide, as in yeast cells, the product is ethanol.

formation of approximately 18-times more ATP than that formed in glycolysis. Oxygen, the final electron-acceptor in the electron transport chain, is reduced to form H_2O (fig. 12.2*a*). Without oxygen to accept electrons passed through the electron transport chain, the chain is not functional and an aerobic organism will quickly die. We can summarize aerobic respiration as follows:

Summary Equation for Aerobic Respiration

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + ATP + Heat$$

Glucose Oxygen Carbon Dioxide Water

Question 1

Why must aerobic organisms such as yourself inhale oxygen and exhale CO_2?

Other organisms called **anaerobes** live without oxygen and may even be killed by oxygen in the atmosphere. Some of these anaerobes are primitive bacteria that gather their energy with a pathway of anaerobic respiration that uses inorganic electron acceptors other than oxygen. For example, many bacteria use nitrate, sulfate, or other inorganic compounds as the electron acceptor instead of oxygen. Other anaerobes use glycolysis, but the pyruvate from glycolysis is reduced via anaerobic **fermentation** to either CO_2 and ethanol (in plants and some microbes such as yeast) or lactic acid (in other microbes and oxygen-stressed muscles of animals; fig. 12.2*b*). We can summarize anaerobic fermentation in figure 12.2*b* in the following equations:

Anaerobic Fermentation in Plants and Some Microbes

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + ATP + Heat$$

Glucose Ethanol Carbon Dioxide

Anaerobic Fermentation in Animals and Some Microbes

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH + ATP + Heat$$

Glucose — Lactic Acid

Notice from these equations that plants (as well as prokaryotes and other eukaryotes such as yeasts) can temporarily conduct anaerobic fermentation that reduces pyruvate from glycolysis to ethanol and carbon dioxide. This occurs, for example, in roots that penetrate anaerobic soils and sediments.

Anaerobic fermentation does not involve or benefit from the additional ATP produced by the citric acid cycle or electron transport chain. Thus, the ability of an organism to live in the absence of oxygen comes at a price: Anaerobic fermentation produces 18-fold less ATP per glucose molecule than does aerobic respiration.

Question 2

What are the advantages and disadvantages of anaerobic fermentation?

In today's exercise, you will study the major features of cellular respiration. Let's begin with a type of anaerobic fermentation with which you are already familiar: alcoholic fermentation by yeast.



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

PRODUCTION OF CO_2 DURING ANAEROBIC FERMENTATION

Yeast are fungi used in baking and producing alcoholic beverages. They can respire in the absence of O_2 and can oxidize glucose to ethanol and CO_2. To demonstrate CO_2 production during anaerobic fermentation by yeast (fermentation), follow procedure 12.1. In this procedure you will observe the effects of these compounds on respiration:

Pyruvate—a product of glycolysis; pyruvate is reduced to ethanol or lactic acid during anaerobic fermentation

Magnesium sulfate ($MgSO_4$)—provides Mg^{2+}, a cofactor that activates some enzymes of glycolysis

Sodium fluoride (NaF)—an inhibitor of some enzymes of glycolysis

Glucose—a common organic molecule used as an energy source for respiration

Procedure 12.1 **Demonstrate CO_2 production during anaerobic fermentation**

1. Label seven test-tubes and add the solutions listed in table 12.1.
2. Completely fill the remaining volume in tubes 1–6 with the yeast suspension that is provided. Fill the remaining volume in tube 7 completely with distilled water.



NaF is a poison. Handle it carefully.

3. For each tube, slide an inverted, flat-bottomed test tube down over the yeast-filled tube (see fig. 1.3). Hold the yeast-filled tube firmly against the inside bottom of the cover tube and invert the assembly. Your instructor will demonstrate how to slide this slightly larger empty tube over the top of each yeast tube and invert the assembly. If done properly, air will not be trapped at the top of the tube of yeast after inversion.
4. Incubate the tubes at 37°C for 40 min. While you are waiting, write your predictions for each tube:

Tube	Predicted Results and Brief Explanation
1.	
2.	
3.	
4.	
5.	
6.	
7.	

5. After 40 min, measure the height (in millimeters) of the bubble of accumulated CO_2. Record your results in table 12.1.
6. The effects of pyruvate, $MgSO_4$, NaF, and glucose on CO_2 production are best determined by comparing each tube to the control rather than by ranking all of the treatment tubes. For each variable in table 12.2, record the number of the tube containing the compound being tested and the number of the tube serving as the control for that compound.

Question 3

a. What was the purpose of tube 7?

b. How was the effect of concentration of inhibitor tested in this experiment? How did the concentration of NaF affect anaerobic fermentation in your experiment? Why?

c. Which compounds listed in step 6 are intermediates in the respiratory pathway?

d. Why did tube 6 produce CO_2 even though an inhibitor of glycolysis was present?

e. Compare tubes 4 and 5. How was CO_2 production affected by the 10-fold increase in the amount of NaF? For example, was it also changed 10-fold?

f. Did magnesium (a cofactor that activates many enzymes) promote respiration? If not, what are some possible reasons?

g. Smell the contents of the tube containing the most CO_2. What compound do you smell?

h. What is the economic importance of fermentation by yeast?

i. What gas is responsible for the holes in baked bread?

TABLE 12.1

EXPERIMENTAL TREATMENTS AND CO_2 PRODUCTION DURING ANAEROBIC FERMENTATION

TUBE	3 M Na PYRUVATE (ACTIVATOR)	0.1 M $MgSO_4$ (ACTIVATOR)	0.1 M NaF (INHIBITOR)	5.0% GLUCOSE (ACTIVATOR)	WATER	FILL WITH	CO_2 PRODUCED AFTER 40 MIN (mm)
1	—	—	—	—	7.5 mL	Yeast suspension	
2	—	—	—	2.5 mL	5.0 mL	Yeast suspension	
3	—	5.0 mL	—	2.5 mL	—	Yeast suspension	
4	—	—	0.5 mL	2.5 mL	4.5 mL	Yeast suspension	
5	—	—	5.0 mL	2.5 mL	—	Yeast suspension	
6	2.5 mL	—	2.5 mL	2.5 mL	—	Yeast suspension	
7	—	—	—	2.5 mL	2.5 mL	Water	

TABLE 12.2

EFFECTS OF FOUR CHEMICAL VARIABLES ON CO_2 PRODUCTION DURING ANAEROBIC FERMENTATION

VARIABLE	TUBE # WITH VARIABLE	TUBE # CONTROL	EFFECT OF VARIABLE ON RESPIRATION RATE	MECHANISM FOR THE EFFECT
Yeast				
Glucose				
NaF				
Na Pyruvate				
$MgSO_4$				

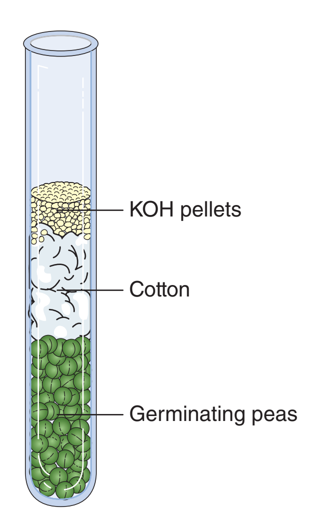


Figure 12.3 Test tube containing germinating peas, cotton, and KOH pellets.

If time and facilities are available, repeat procedure 12.1 and incubate the tubes at 4°C (refrigerator), 20°C (incubator), and/or 55°C (incubator). Use your data to explain the effect of temperature on fermentation by yeast.

OXYGEN CONSUMPTION DURING AEROBIC RESPIRATION

Aerobic respiration uses oxygen as the terminal electron acceptor in the electron transport chain. Because this oxygen is reduced to water, you can measure aerobic respiration by measuring the consumption of oxygen. During respiration, CO_2 is produced, while O_2 is consumed. Review the summary equation for aerobic respiration in the introduction of this exercise. In the following experiment, KOH is used to absorb the CO_2. Therefore, the net change in gas volume is a measure of oxygen consumption.

Procedure 12.2 **Determine oxygen consumption during aerobic respiration (may be done as a demonstration)**

1. Fill a test tube or flask half-full with germinating peas and another half-full with heat-killed peas. The germinating peas have been soaked in water in the dark for three to four days.

2. Cover the contents of each tube with a loose-fitting plug of cotton.

3. Cover the cotton with approximately 1 cm of loosely packed pellets of potassium hydroxide (KOH) (fig. 12.3).



Potassium hydroxide (KOH) is a strong, caustic base. Handle it carefully. If you get any KOH on your skin, rinse immediately with water.

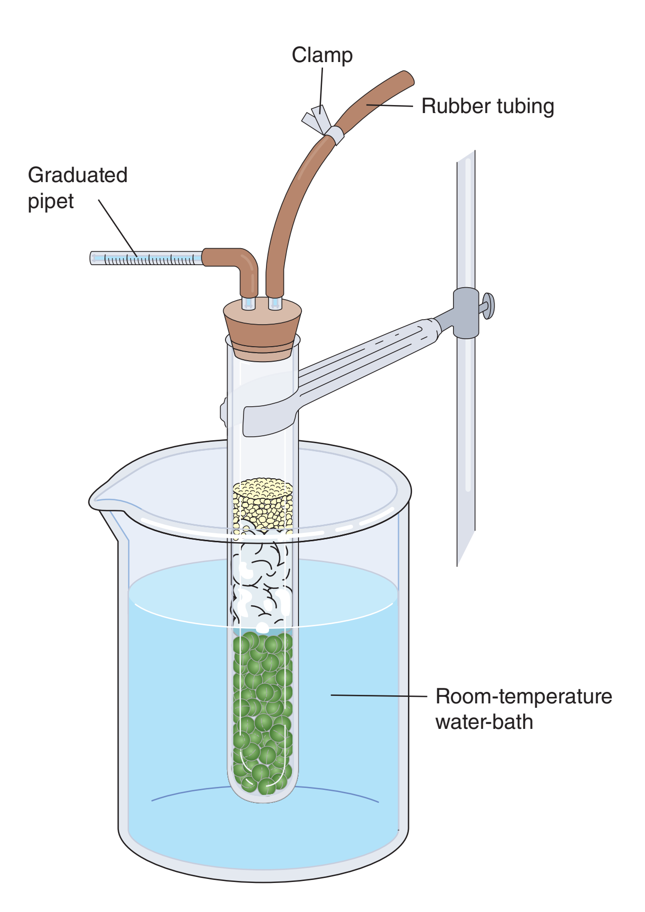


Figure 12.4 Test tube with stopper having capillary tubes attached. The tube will be covered in foil.

4. Place a stopper containing a capillary tube or graduated pipet with an attached outlet tube into both tubes containing peas (fig. 12.4). The capillary tube or graduated pipet should be oriented horizontally.

5. Cover the tube with foil to prevent light and photosynthesis.

6. Vertically clamp the tubes to a ring stand so that the bottom of each tube is submerged in a room-temperature water-bath. The water-bath will minimize temperature fluctuations in the tube.

7. Use a Pasteur pipet to inject enough dye into each capillary tube so that approximately 1 cm of dye is drawn into each capillary tube. The capillary tube or graduated pipet should be oriented horizontally.

8. After waiting 1 min for equilibration, attach a pinch clamp to the outlet tube and mark the position of the dye with a wax pencil. Write your predicted results and a brief explanation here:

TABLE 12.3

OXYGEN CONSUMPTION BY SEEDS AT THREE TEMPERATURES

		mL O_2 Consumed					
		10 min		20 min		30 min	
Treatment	0 min	Alive	Heat-Killed	Alive	Heat-Killed	Alive	Heat-Killed
Room temperature	0	________	________	________	________	________	________
Ice bath	0	________	________	________	________	________	________
Warm water-bath	0	________	________	________	________	________	________

9. Use a wax pencil to mark the position of the dye every 10 min for the next 30 min.

10. After each time interval, measure the distance the dye moved from its starting point; record your data in table 12.3.

11. Remove the pinch clamp from the outlet valve and return the dye to the end of the capillary tube by tilting the capillary tube.

12. Repeat steps 1–11 using tubes incubated in an ice bath and warm (35°C) water-bath. You can save time by running all of these treatments simultaneously. Record your results in table 12.3.

Question 4

a. What was the purpose of adding heat-killed peas to a tube?

b. In which direction did the dye move? Why?

c. What does this experiment tell you about the influence of temperature on oxygen consumption during cellular respiration?

PRODUCTION OF CO_2 DURING AEROBIC RESPIRATION

CO_2 produced during cellular respiration can combine with water to form carbonic acid:

$$\underset{\text{Carbon dioxide}}{CO_2} + \underset{\text{Water}}{H_2O} \leftrightarrow \underset{\text{Carbonic acid}}{H_2CO_3}$$

In this procedure (fig. 12.5), you will use phenolphthalein to detect changes in pH resulting from the production of CO_2 (and, therefore, carbonic acid) during cellular respiration. Phenolphthalein is red in basic solutions and colorless in acidic solutions. Thus, you can monitor cellular respiration by measuring acid production as change in pH. **pH** is a measure of the acidic or basic properties of a solution; pH 7 is neutral. Solutions having a pH < 7 are acidic, and solutions having a pH > 7 are basic (see Exercise 5).

In procedure 12.3, you will not directly measure the volume of CO_2 produced by a respiring organism. Instead, you will measure the volume of NaOH used to neutralize the carbonic acid produced by the CO_2, and thereby calculate a relative measure of respiration.

Question 5

The organisms you will study include an animal (snail) and a plant (*Elodea*). Which do you think will respire more? Write your hypothesis here:

Procedure 12.3 **Measure relative CO_2 production by aerobic organisms**

Experimental Setup

1. Obtain 225 mL of culture solution provided by your instructor. This solution has been dechlorinated and adjusted to be slightly acidic.

2. Place 75 mL of this solution in each of three labeled beakers (fig. 12.5).

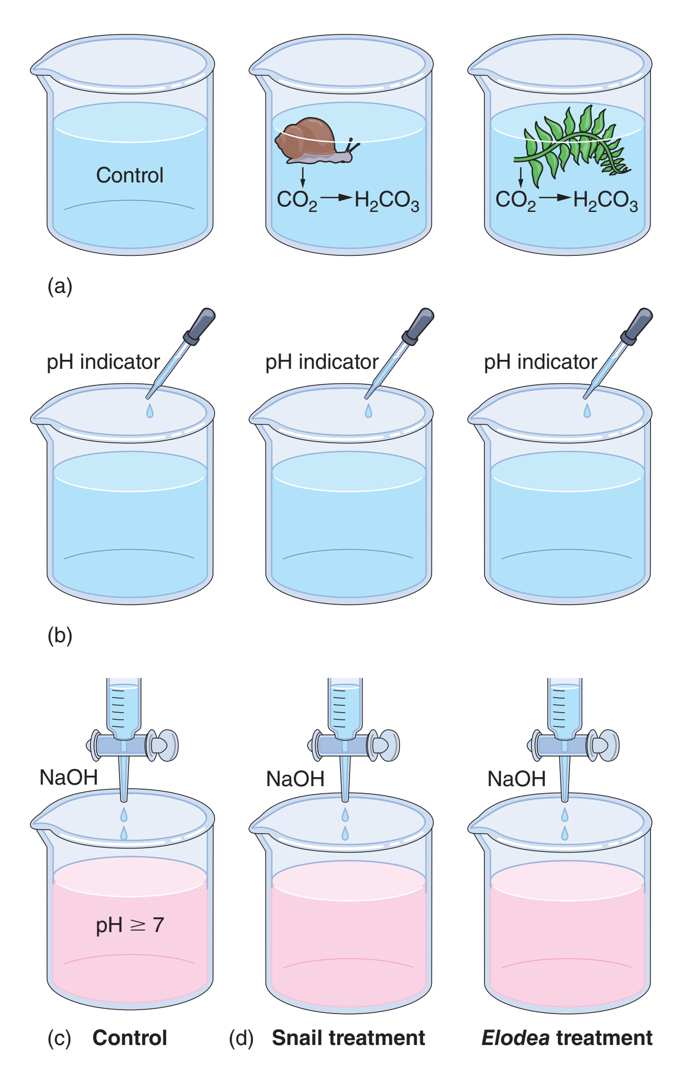


Figure 12.5 The procedure to determine the relative respiration rates of a plant and animal. (*a*) During respiration, organisms release CO_2, which combines with water to form carbonic acid (H_2CO_3). (*b*) The acidic solutions remain colorless after addition of phenolphthalein, a pH indicator. (*c*) Titration of the control with NaOH (a base) will make the solution basic and pink when the pH reaches the end-point of phenolphthalein. (*d*) The treatment solutions are then titrated to the pink end-point matching the control. The volume of NaOH needed to reach the end-point indicates the relative amounts of dissolved CO_2 produced during respiration.

3. Obtain the organisms listed in table 12.4 from your instructor and determine the volume of each organism by following steps 4–6. Your instructor may substitute a small fish for the snail.

Determine Volume by Water Displacement

4. Place exactly 25 mL of water in a 50-mL graduated cylinder.
5. Place the organism in the cylinder and note the increase in volume above the original 25 mL. This increase equals the volume of the organism.
6. Record the volumes in table 12.4. Gently place similar masses of each plant or animal in the appropriate beaker.

Incubate Experimental Treatments

7. Cover each beaker with a plastic film or petri dish top and set them aside on your lab bench. Place the beaker containing the *Elodea* in the dark by covering it with a coffee can or aluminum foil.
8. Allow the organisms to respire for 15 min.
9. Gently remove the organisms from the beakers and return them to their original culture bowls.

Titrate to Gather Your Raw Data

10. Add four drops of phenolphthalein to the contents of each beaker. The solutions should remain clear because the solutions are acidic.
11. Obtain a burette or dropper bottle to dispense NaOH (2.5 mM). Add NaOH drop by drop to the contents of the control beaker. Thoroughly mix the contents of the beaker after adding each drop. Record in table 12.4 the milliliters of NaOH required to reach the end point of phenolphthalein. The end-point is when you first notice that the solution is pink.
12. Repeat step 11 for beaker 1; be sure to add NaOH only until the solution is the same shade of pink as the control beaker. Record the number of milliliters of NaOH added to beaker 1 in table 12.4.
13. Repeat step 11 for beaker 2.

Table 12.4

Data for Measuring CO_2 Production during Respiration

Organisms	Total Volume of Organisms (mL)	Milliliters of NaOH to Reach End-Point (mL NaOH)	Relative Respiration Rate of Organisms (mL NaOH)	Respiration Rate per Milliliter of Organism (mL NaOH/mL organisms)
Beaker 1: 4 snails	________	________	________	________
Beaker 2: *Elodea*	________	________	________	________
Control beaker	0	________	0	0

Calculate Your Results

14. For beaker 1, determine the relative respiration rate for organisms by subtracting the milliliters NaOH added to the control beaker from the milliliters NaOH added to beaker 1. Record this value in table 12.4.

15. Repeat step 14 for beaker 2.

16. For beakers 1 and 2, determine the respiration rate per milliliter of organism by dividing the relative respiration rate for organisms by the volume of the organism(s). Record these values in table 12.4.

Question 6

a. In this exercise you measured the relative respiration rates of an animal and a plant. Why were you cautioned about having no algae in the control beaker?

b. Before you gathered your raw data, you formulated a hypothesis about the expected results. After considering your data, do you accept or reject your hypothesis? Why?

c. What is your major conclusion from the results of this procedure?

d. What features of the biology of the organisms that you used most likely contributed to the observed differences in respiration rate?

e. Do you feel justified in drawing conclusions from your work about all plants and animals? Or only about snails and *Elodea?* Why?

f. How would you expand this experiment to further test your conclusions about other plants and other animals?

g. What other organisms might you include in an expanded experiment? Why did you choose these organisms?

DEMONSTRATION: DETERMINING THE METABOLIC RATE OF A MOUSE

The rate of O_2 uptake during cellular respiration indicates the metabolic rate of an organism. In procedure 12.4 you will measure O_2 uptake by measuring changes in air pressure as O_2 is removed from the air by a respiring mouse. Changes in air pressure can be attributed primarily to O_2 consumption (rather than CO_2 production or exhalation of water vapor) only if exhaled CO_2 and H_2O are removed from the air. This is accomplished by adding ascarite (which adsorbs CO_2) and drierite (which adsorbs H_2O) to the experimental setup (fig. 12.6). Use procedure 12.4 to estimate the metabolic rate of a mouse.

Procedure 12.4 Estimate the metabolic rate of a mouse

1. Weigh a mouse to the nearest 0.1 g. Record this weight in table 12.5 and place the mouse in the jar of a respirometer (fig. 12.6). Use a fan to circulate air in the jar and allow the mouse to get accustomed to the jar.

2. Attach a 10-mL syringe filled with air to the respirometer.

3. Seal the respirometer jar with a lid. Then close the air escape line with a clamp and record the position of the dye solution in the right column of the curved capillary tube. This tube is called a manometer.

4. Inject 10 mL of air into the respirometer. The level of dye in the right side of the manometer will rise because of the increased presence of air. After injecting the 10 mL of air into the respirometer, record the position of the dye solution in table 12.5.

5. Allow the mouse to respire. The air pressure in the respirometer should decrease as O_2 is consumed, and the dye level in the right column of the manometer should decrease.

6. Record in table 12.5 the elapsed time for the dye level to return to its original position. This is the time for the mouse to consume 10 mL of O_2. Record this time as "A" in the Calculations section of table 12.5.

7. Gently return the mouse to its cage.

8. Calculate the number of liters consumed by the mouse per day by using the following formula:

Liters of O_2 consumed per day =
1440 minutes per day ÷ minutes
to consume 1 liter of O_2

Record this as "B" in the Calculations section of table 12.5.

9. Calculate and record in table 12.5 the mouse's metabolic rate in kcal/day, assuming that 4.8 kcal of energy are used for each liter of O_2 consumed. Record this as "C" in the Calculations section of table 12.5.

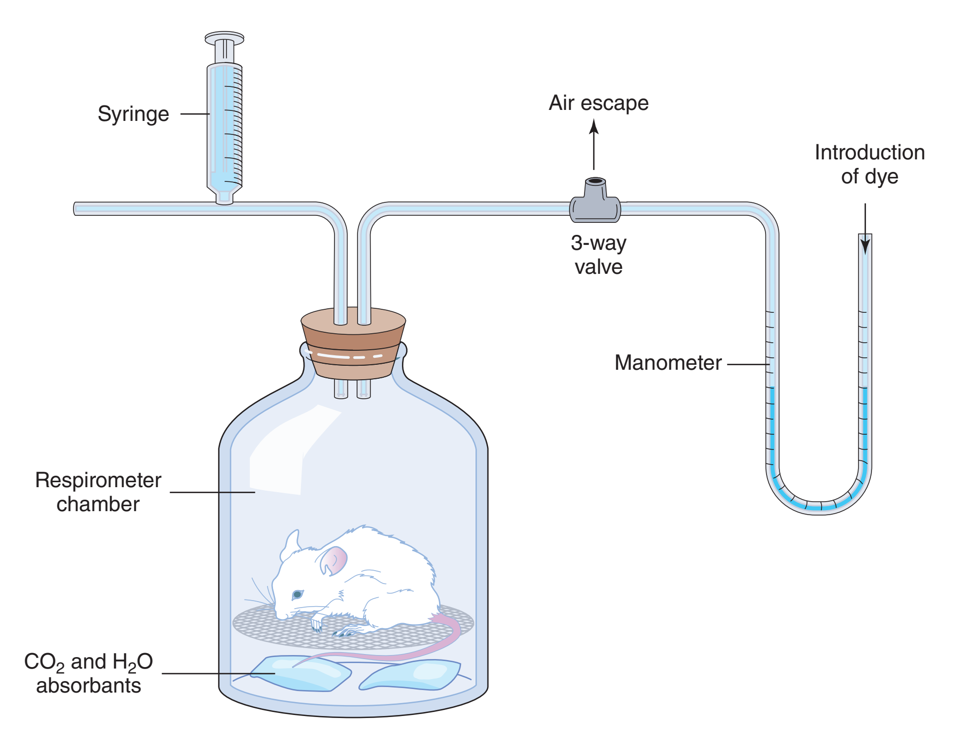


Figure 12.6 Respirometer with mouse.

TABLE 12.5

DATA FOR DETERMINATION OF METABOLIC RATE OF A RESPIRING MOUSE

OBSERVATIONS AND DATA

Weight of mouse: ________ grams

Initial position of dye solution: ________

Position of dye solution after injection of 10 mL of air: ________

Minutes for dye level to return to initial position: (minutes per 10 mL oxygen): ________ min

CALCULATIONS

A	Minutes to consume 1 liter of O_2 = (minutes per 10 mL oxygen) × 100 = ________ min
B	Liters of O_2 consumed per day = 1440 minutes per day ÷ A = ________ liters per day
C	Experimental metabolic rate as kcal per day = B × 4.8 kcal per liter O_2 = ________ kcal per day
D	Predicted metabolic rate = 70 × (weight of mouse)$^{3/4}$ = ________ kcal per day

10. Calculate and record the predicted metabolic rate obtained from the following general equation for metabolic rate of small mammals:

Predicted metabolic rate = 70 × (body weight in kg)$^{3/4}$

Record this rate as "D" in the Calculations section of table 12.5.

11. Compare your experimental value with the predicted value for metabolic rate.

Question 7

a. Is the predicted metabolic rate similar to that which you determined experimentally?

INVESTIGATION

The Effect of Environmental Stimuli on Cellular Respiration

Observations: Respiration, like all biochemical processes, responds to environmental stimuli (e.g., temperature, salinity, acidity, light). However, some organisms tolerate a wider range of conditions than others.

Question: How is the rate of cellular respiration affected by environmental stimuli?

a. Establish a working lab group and obtain Investigation Worksheet 12 from your instructor.

b. The preceding question will give you a general direction for your work, but you'll need to refine it before proceeding. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Review procedures 12.1 and 12.3, which use yeast, snails, and *Elodea* as model organisms to investigate respiration. Outline on Worksheet 12 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

b. What could cause any differences in these values?

c. Determine the metabolic rate of other organisms available in the lab. How do their metabolic rates compare with that of a mouse?

APPLICATIONS OF ANAEROBIC RESPIRATION

Making Wine

In this exercise, you've seen how easy it is to demonstrate alcoholic fermentation by yeast. Many biologists as well as nonbiologists use this reaction to make their own wine. If you're game for an introduction to home wine-making, try the following procedure.

Figure 12.7 Experimental setup for making wine.

Procedure 12.5 **Making wine**

1. Thoroughly clean and sterilize all glassware.

2. Combine a cake of yeast with either bottled grape juice or cranberry juice. Mix the yeast and juice in a ratio of approximately 5 liters of juice to 1 gram of yeast.

3. Add approximately 650 mL of the juice-yeast mix to each of four 1-liter Erlenmeyer flasks (or use 1- to 2-liter recycled plastic pop bottles).

4. Dissolve the following amounts of sucrose in each flask:

Flask 1: 75 g	Flask 3: 300 g
Flask 2: 150 g	Flask 4: no sucrose

5. For each flask, set up the fermentation apparatus as shown in figure 12.7.

6. Be sure to keep the procedure anaerobic by keeping the end of the exit tube under water in the adjacent flasks. This will prevent contamination by airborne bacteria and yeast.

7. Incubate the flasks at temperatures between 15°C and 22°C. Although fermentation will continue for a month or so, most fermentation will occur within the first 14 days. Fermentation is complete when bubbling stops.

8. To test your wine, remove the stopper and use a piece of tubing to siphon off the wine solution without disturbing the sediment in the bottom of the flasks. You may then want to filter the solution to remove any remaining yeast cells from the wine.

9. Taste your wine. If your wine has been contaminated by bacteria that produce acetic acid, vinegar may have been formed, so take your first sip cautiously.

Question 8

a. What differences are there in wines produced with different amounts of sugar?

b. What would happen if oxygen were present (i.e., if conditions were not anaerobic)? If you have time, test your hypothesis.

1. How would you modify the experimental setup to introduce oxygen?

2. What results would you predict?

3. Based on your results, was your hypothesis accurate? Explain.

If you're interested in the finer points of wine making, visit your local bookstore or library. There may also be a local society of amateur wine-makers in your area who will be glad to give you some pointers on creating "a simply delightful bouquet."

Making Kimchee

Pickling is an ancient way of preserving food. Pickling involves the anaerobic fermentation of sugars to lactic acid; this acid lowers the pH of the medium, thereby creating an environment in which other food-spoiling organisms cannot grow. Common foods preserved with pickling include sauerkraut, yogurt, and dill pickles. The ancient Chinese cabbage product kimchee, still a major part of the Korean diet, is also made with pickling. Here's how to make kimchee.

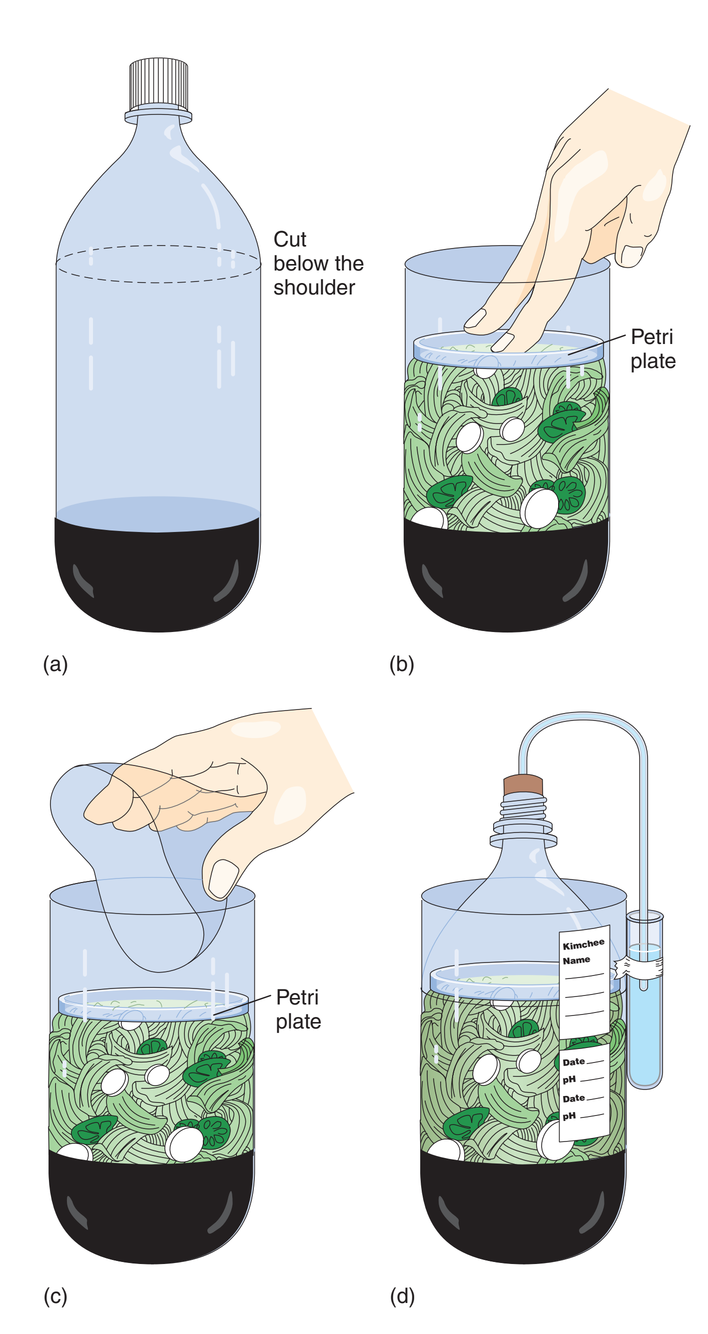


Figure 12.8 Experimental setup for making kimchee. See the text for the recipe and procedure.

Procedure 12.6 Making kimchee

1. Coarsely shred a head of cabbage. Place it in a mixing bowl with salt and allow it to wilt. This will draw some of the liquid out and prevent the finished kimchee from being watery.

2. Cut a 2-liter bottle just below the shoulder, as shown in figure 12.8*a*.

3. Add alternating layers of cabbage, garlic, pepper, and a sprinkling of salt in the bottle, pressing each layer down until the bottle is full. If you're using chilies or pepper, do not touch your eyes or mouth.

4. Place the lid, rim side up, atop the ingredients. Press down (fig. 12.8*b*). Within a few minutes, the salt will draw liquid from the cabbage; that liquid will begin to accumulate in the bottle.

5. For the next hour or so, continue to press the cabbage. You should then be able to fit the bottle top inside the bottle bottom, forming a sliding seal (fig. 12.8*c*). When you press with the sliding seal, cabbage juice will rise above the petri plate and air will bubble out around the edge of the plate.

6. The cabbage will pack half to two-thirds of the bottle's volume (fig. 12.8*d*). Every day, press on the sliding seal to keep the cabbage covered by a layer of juice.

Question 9
What happens when you press on the cabbage? How do you explain this?

7. Use pH-indicator paper to measure and record the pH of the juice each day (see Exercise 5).

8. After 4 to 7 days (depending on the temperature), the pH will have dropped from about 6.5 to about 3.5. Enjoy your kimchee!

Questions for Further Thought and Study

1. What is the difference between respiration and breathing?

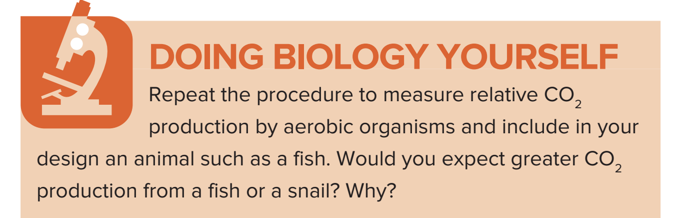
2. Does cellular respiration occur simultaneously with photosynthesis in plants? How could you determine the relative rates of each?

3. What role does cellular respiration play in the metabolism of an organism?

4. What modifications of cellular respiration might you expect to find in dormant seeds?

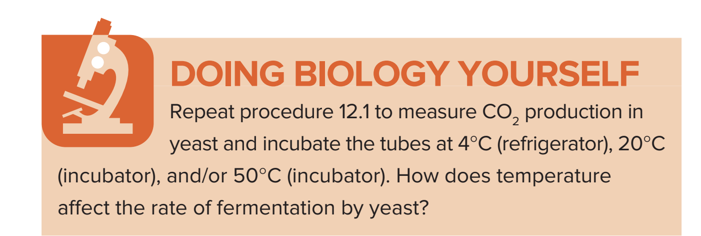
5. In procedure 12.3, why did you subtract the control value from the titrant in beaker 1 and beaker 2?

6. Why is the volume of CO_2 production rather than O_2 uptake an adequate measure of respiration for the study of respiration rate?



DOING BIOLOGY YOURSELF

Repeat the procedure to measure relative CO_2 production by aerobic organisms and include in your design an animal such as a fish. Would you expect greater CO_2 production from a fish or a snail? Why?



DOING BIOLOGY YOURSELF

Repeat procedure 12.1 to measure CO_2 production in yeast and incubate the tubes at 4°C (refrigerator), 20°C (incubator), and/or 50°C (incubator). How does temperature affect the rate of fermentation by yeast?

Photosynthesis

Pigment Separation, Starch Production, and CO_2 Uptake



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Figure 13.1 The energy that drives photosynthesis comes from the sun. Less than 1% of all the energy that reaches the earth from the sun is captured by photosynthesis. This 1% fuels virtually all life on earth.

Learning Objectives

By the end of this exercise you should be able to:

1. Relate each part of the summary equation for photosynthesis to the synthesis of sugar.
2. Describe the differences between the light-dependent and light-independent reactions involved in photosynthesis.
3. Separate the photosynthetic pigments using paper chromatography and calculate their R_f numbers.
4. Use a spectroscope to describe the absorption of visible light by chlorophyll.
5. Describe fluorescence.
6. Describe the process of electron transport in chloroplasts and its role in photosynthesis.
7. Describe the change of pH that occurs as plants take up CO_2 from their environment during photosynthesis.
8. Describe the distribution of starch in leaves resulting from photosynthesis relative to the amount of light they receive and the distribution of pigments.



Please visit connect.mheducation.com to review online resources tailored to this lab.

Photosynthesis is the most important series of chemical reactions that occurs on earth (fig. 13.1). Indeed, virtually all life depends on photosynthesis for food and oxygen. **Photosynthesis** is a complex chemical process that converts radiant energy (light) to chemical energy (sugar). The following equation summarizes photosynthesis:

$$\underset{\text{Carbon dioxide}}{6\,CO_2} + \underset{\text{Water}}{12\,H_2O} \xrightarrow[\text{chlorophyll}]{\text{light}} \underset{\text{Sugar}}{C_6H_{12}O_6} + \underset{\text{Water}}{6\,H_2O} + \underset{\text{Oxygen}}{6\,O_2}$$

Thus, photosynthesis is the light-dependent and chlorophyll-dependent conversion of carbon dioxide and water to sugar, water, and oxygen. Oxygen is released to the environment, and sugar is used to fuel growth or is stored as starch, a polysaccharide. Although water is present on both sides of the summary equation, these are not the same water molecules. The "reactant" water molecules (i.e., those on the left side of the equation) are split to release electrons during the photochemical (i.e., light-dependent) reactions. The "product" water molecules (i.e., those on the right side of the equation) are assembled from hydrogen and oxygen released during the photochemical and biochemical (i.e., light-independent) reactions. The photochemical reactions of photosynthesis are often referred to as the "light reactions." The biochemical reactions are often referred to as the "dark reactions" or the Calvin cycle, in honor of Melvin Calvin, the botanist who described the reactions.

As mentioned, photosynthesis can be divided into two sets of reactions (fig. 13.2). Some characteristics of these reactions are compared here:

Photochemical "Light" Reactions	***Biochemical "Dark" Reactions***
Fast (practically instantaneous)	Slower, but still extremely fast
Light-dependent	Light-independent
Splits water to release oxygen, electrons, and protons	Converts (fixes) carbon dioxide to sugar

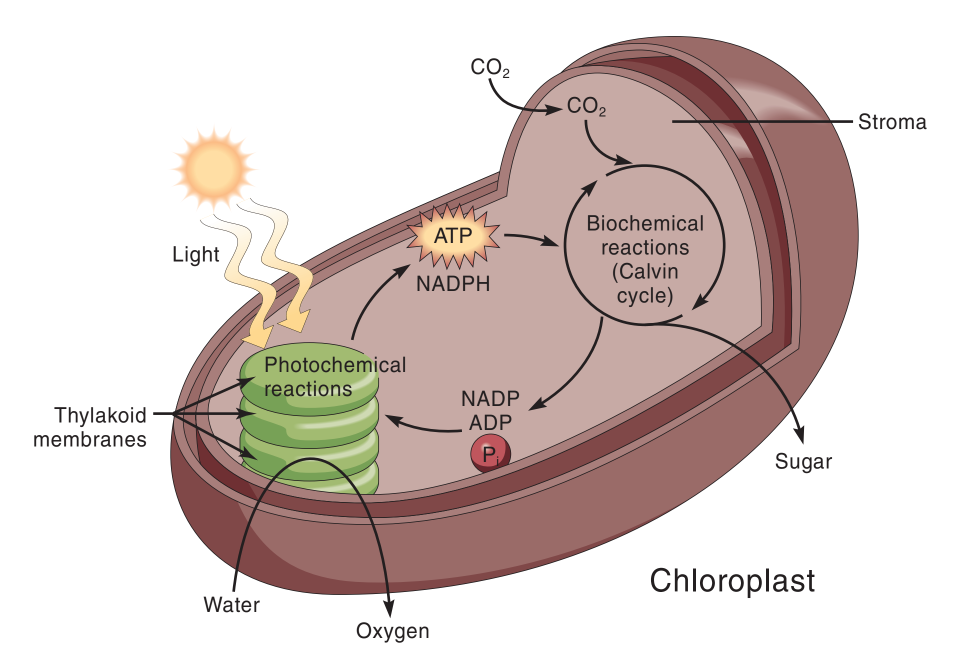


Figure 13.2 Photosynthesis occurs in chloroplasts and consists of photochemical (the light-dependent "light reactions") and biochemical (the light-independent "dark reactions" including the Calvin cycle) reactions. The photochemical (i.e., light) reactions convert light-energy to chemical energy captured in ATP and NADPH. The biochemical (i.e., dark) reactions use the ATP and NADPH produced by the photochemical reactions to reduce CO_2 to sugars. The photochemical reactions occur on thylakoid membranes, whereas the biochemical reactions occur in the stroma.

In today's exercise, you'll investigate some of the major aspects of photosynthesis, beginning with the isolation and identification of photosynthetic pigments.

Before you begin studying photosynthesis, we should remind you that *all* organisms (including plants) carry out respiration in one form or another, but chlorophyll-containing organisms can *also* photosynthesize.

PAPER CHROMATOGRAPHY OF PHOTOSYNTHETIC PIGMENTS

Light must be absorbed before its energy can be used. A substance that absorbs light is a **pigment.** The primary photosynthetic pigments that absorb light for photosynthesis are **chlorophylls *a*** and ***b*.** However, chlorophylls are not the only photosynthetic pigment; **accessory pigments** such as **carotenoids** and **xanthophylls** also absorb light and transfer energy to chlorophyll *a.*

Paper chromatography is a technique for separating dissolved compounds such as chlorophyll, carotene, and xanthophyll. When a solution of these pigments is applied to strips of paper, the pigments adsorb onto the fibers of the paper. When the tip of the paper is immersed in a solvent, the solvent is absorbed and moves up through the paper. As the solvent moves through the spot of applied pigments, the pigments dissolve in the moving solvent. However, the pigments do not always keep up with the moving solvent—some pigments move almost as fast as the solvent, whereas others move more slowly. This differential movement of pigments results from each pigment's solubility and characteristic tendency to stick (i.e., be adsorbed) to the cellulose fibers of the paper. A pigment's molecular size, polarity, and solubility determine the strength of this tendency; pigments adsorbed strongly move slowly, whereas those adsorbed weakly move fastest. Thus, each pigment has a characteristic rate of movement, and the pigments can be separated from each other. In procedure 13.1, four bands of color will appear on the strip—a yellow band of xanthophylls, a yellow-orange band of carotenes, a blue-green band of chlorophyll *a,* and a yellow-green band of chlorophyll *b.*

The relationship of the distance moved by a pigment to the distance moved by the solvent front is specific for a given set of conditions. We call this relationship the R_f number and define it as follows:

$$R_f = \frac{\textbf{Distance moved by pigment}}{\textbf{Distance from pigment origin to solvent front}}$$

Paper chromatography can be used to identify each pigment by its characteristic R_f. This R_f is constant for a given pigment in a particular solvent-matrix system.



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 13.1 Separate plant pigments by paper chromatography

1. Observe the contents of the container labeled "Plant Extract." You'll use paper chromatography to separate its pigments.



Extinguish all hotplates and flames before you do this experiment. Keep all solvents away from hotplates and flames at all times.

Question 1
What color is the plant extract, and why is it this color?

2. Obtain a strip of chromatography paper from your lab instructor. Handle the paper by its edges so that oil on your fingers does not contaminate the paper.
3. Use a Pasteur pipet or a fine-tipped brush to apply a stripe of plant extract approximately 2 cm from the tip of the paper (fig. 13.3). Blow the stripe dry and repeat this application at least 15 times. For this separation to work well, you must start with an extremely concentrated application of extract on the paper.
4. An alternate procedure is to place a fresh leaf directly on the paper, then press and roll the edge of a coin (quarter) over the leaf to crush the cells and form a stripe of pigment.
5. Place the chromatography strip in a test tube containing 2 mL of chromatography solvent (9 parts petroleum ether : 1 part acetone). Position the chromatography strip so that the tip of the strip (but not the stripe of plant extract) is submerged in the solvent. You can do this by hooking the strip of paper with a pin inserted in the tube's stopper (fig. 13.4).
6. Place the tube in a test-tube rack and watch as the solvent moves up the paper. Keep the tubes capped and undisturbed during solvent movement.
7. Remove the chromatography strip when the solvent front is within 1 cm of the top of the strip (i.e., after 2–3 min). Mark the position of the solvent front with a pencil and set the strip aside to dry. Observe the bands of color, then draw your results on figure 13.5. Use your textbook or other materials in lab to identify the different bands of pigments according to their position and color. For example, xanthophylls appear yellow.
8. Use a ruler to measure the distance from the pigment origin to the solvent front and from the origin to each pigment band. Calculate the R_f number for each pigment; record your data in table 13.1.

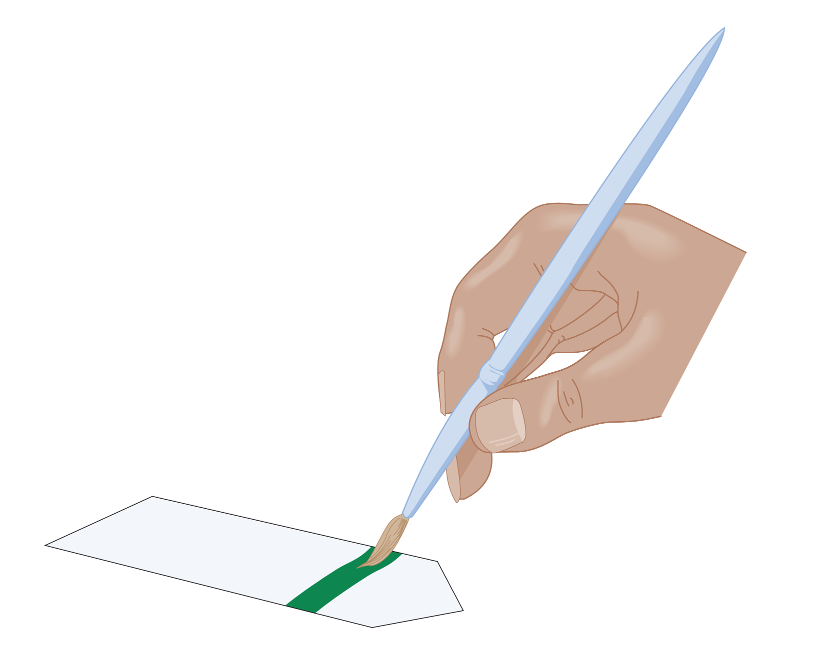


Figure 13.3 Application of pigment extract to a chromatography strip.

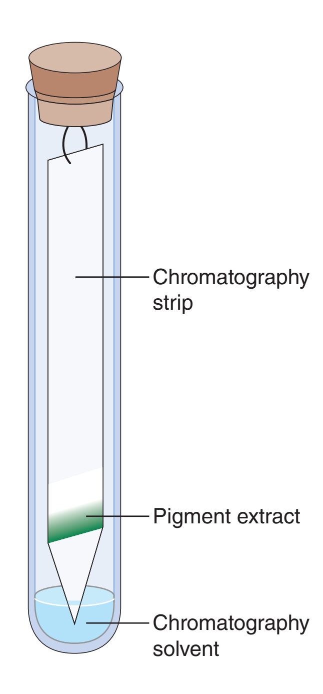


Figure 13.4 Chromatography setup.

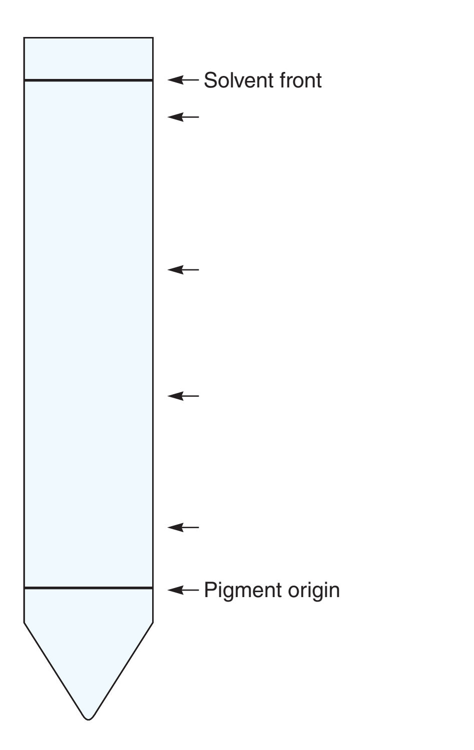


Figure 13.5 Completed chromatogram. On the chromatogram, indicate the color of the band of pigment to the left of the arrows. To the right of the arrows, write the name of the pigment.

TABLE 13.1

R_F NUMBERS FOR FOUR PLANT PIGMENTS

PIGMENT	R_f
Carotene	
Xanthophyll	
Chlorophyll *a*	
Chlorophyll *b*	

Question 2

a. What does a small R_f number tell you about the characteristics of the moving molecules?

b. Which are more soluble in the chromatography solvent, xanthophylls or chlorophyll *a*? How do you conclude this?

c. Would you expect the R_f number of a pigment to change if you altered the composition of the solvent? Why or why not?

d. If yellow xanthophylls were present in the extract, why did the extract appear green?

e. Is it possible to have an R_f number greater than 1? Why or why not?

ABSORPTION OF LIGHT BY CHLOROPHYLL

A **spectroscope** is an instrument that separates white light into its component colors. These colors range from red to violet and appear as a spectrum when separated (fig. 13.6). Observe this spectrum by looking through the spectroscope provided in the lab. Now insert a chlorophyll sample between the light and spectroscope, and observe the resulting spectrum. Light not visible through the extract has been absorbed.

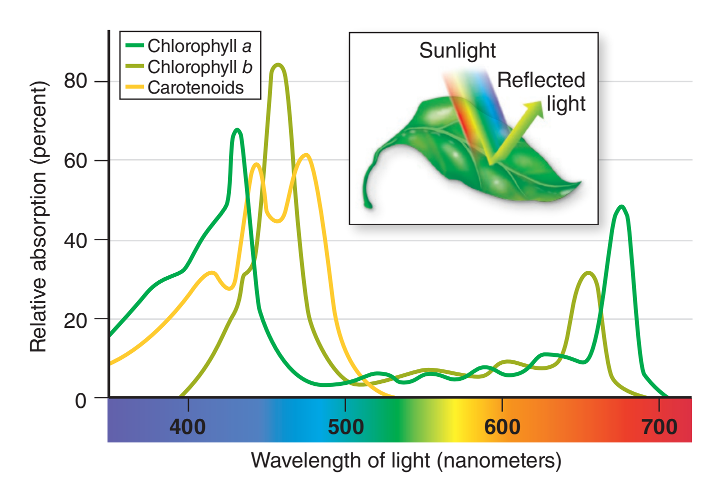
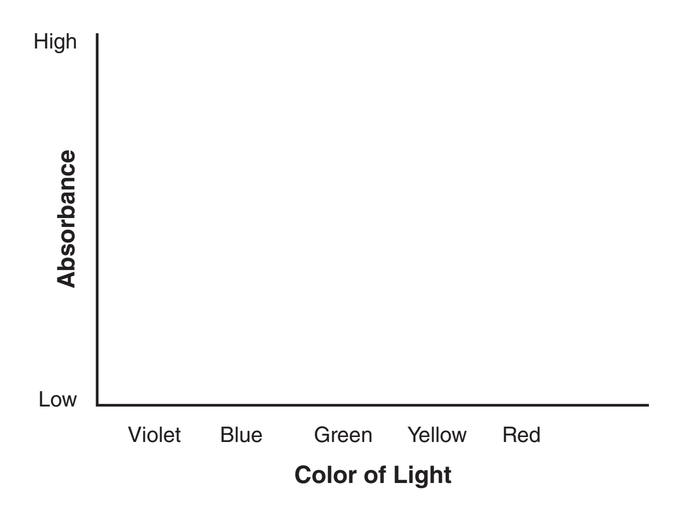


Figure 13.6 The absorption spectrum of chlorophyll and carotenoids. The peaks represent wavelengths of sunlight absorbed by the two common forms of photosynthetic pigment, chlorophylls *a* and *b*, and by the carotenoids. Chlorophylls absorb predominantly violet-blue and red light in two narrow bands of the spectrum, and reflect green light in the middle of the spectrum. Carotenoids absorb mostly blue and green light and reflect orange and yellow light.

Question 3
What colors are diminished or absent?

Based on this observation, complete the following absorption spectrum for chlorophyll. For each color, estimate the relative absorbance of that color by placing an X above the color name at the appropriate position along the *y*-axis. Connect the X's for all colors to complete the absorption spectrum.



Question 4

a. What color of light would be least effective for plant photosynthesis? Why?

b. If available, use an extract from red or orange peppers to plot an absorption spectrum for carotenoids. What colors of light are absorbed by carotenoids? What is the significance of this?

FLUORESCENCE

Light produces reactions only if it is absorbed by a molecule. When sunlight strikes a plant, the chlorophyll absorbs some of the light and reflects some of the light. The green light is reflected and is responsible for the plant's green color. The absorbed light "excites" the chlorophyll by boosting electrons to a higher-energy orbital. During photosynthesis, the energy of these excited electrons from chlorophyll and chlorophyll's central magnesium atom is passed efficiently to another pigment molecule and photosynthesis proceeds. However, to easily observe these energized electrons, we can disrupt the photosynthetic system by blending the cells during the preparation of the plant extract. The chlorophyll electrons in the extract are still energized if you shine light on them, but they are left with nowhere to go. They quickly release their energy by falling back to their original orbitals rather than continuing photosynthesis. As they fall back, they emit a photon of red light. This release of light energy is **fluorescence.** The wavelength of reemitted light is determined by the structure of the molecule reemitting the light.

Procedure 13.2 **Observe fluorescence by chlorophyll**

Place a glass test tube containing chlorophyll extract in front of a bright light. View the extract from the side. (If a UV light is available, you can use the thin-layer chromatography strip from procedure 13.1 to observe fluorescence.)

Question 5
What color light does the extract fluoresce?

ELECTRON TRANSPORT IN CHLOROPLASTS

The photochemical reactions of photosynthesis transfer electrons among various compounds within chloroplasts (fig. 13.7). In 1937, Robin Hill demonstrated that isolated



Figure 13.7 These photosynthetic cells of a moss are packed with bright-green chloroplasts (1000×).

chloroplasts could transfer electrons in the absence of CO_2 if provided with an alternate or artificial electron-acceptor. This observation indicated that electron transport does not require CO_2-fixation to occur. That is, electron transfer and CO_2-fixation involve separate sets of reactions.

You can detect electron transfer using a dye called 2,6-dichlorophenol-indolephenol (DCPIP). In its oxidized state, DCPIP is blue. After accepting electrons, DCPIP becomes reduced and colorless. DCPIP can accept electrons released in chloroplasts during photosynthesis. The rate of DCPIP decoloration depends on its concentration and the rate of electron flow. By measuring decoloration of DCPIP we can indirectly measure the rate of some reactions of photosynthesis. Because the rates of many chemical reactions are pH-dependent, a constant pH of approximately 6.5 is necessary for this experiment. The phosphate buffer used in this experiment maintains a constant pH of the incubation mixture.

Procedure 13.3 **Observe electron transport in chloroplasts**

1. Prepare test tubes according to table 13.2. Metabolically active chloroplasts will be provided by your instructor.
2. Mix the contents of each tube well and place tubes 1–3 approximately 15 cm in front of a high-intensity lightbulb. Wrap tube 4 in aluminum foil and place it with the other three tubes. Do not position tubes behind each other. Keep all tubes directly in the path of the light.
3. Observe the contents of the tubes intermittently; describe the changes in color that you see.
4. If you have time, prepare a replicate of tube 2 in which water is replaced by 1 mL of 0.1 mM simizane or monuron, both herbicides. Handle all herbicides and pesticides carefully.

Question 6

a. What was the purpose of each of the tubes used in this experiment? Which tubes were controls?

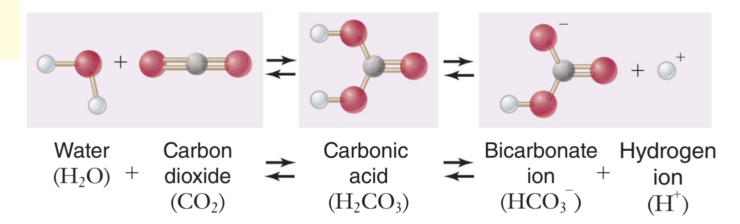
b. What happens when you illuminate the tube containing herbicide?

c. Based on this result, what do you think is the mode of action of these herbicides?

UPTAKE OF CARBON DIOXIDE DURING PHOTOSYNTHESIS

Phenol red (phenol-sulfonphthalein) is a pH-indicator that turns yellow in an acidic solution (pH < 7) and becomes red in a neutral to basic solution (pH > 7). (For more about pH and pH indicators, see Exercise 5.) In this experiment you will use the pH-indicator phenol red to detect the uptake of CO_2 by a photosynthesizing aquatic plant, *Elodea* (see fig. 4.6). Recall that plants use CO_2 during the light-independent reactions of photosynthesis.

To detect CO_2 uptake you will put a plant into an environment that you have made slightly acidic with your breath. Carbon dioxide in your breath will dissolve in water to form carbonic acid, which lowers the pH of the solution:



As the plant fixes CO_2 the pH rises. When the pH rises above 7, the solution turns red.

TABLE 13.2

SOLUTIONS FOR COMPARISON OF PHOTOSYNTHETIC REACTION RATES

Tube	Chloroplasts	0.1 M PO_4 Buffer (pH 6.5)	H_2O	0.2 mM DCPIP
1	0.5 mL	3 mL	1.5 mL	0
2	0.5 mL	3 mL	0.5 mL	1 mL
3	0	3 mL	1.0 mL	1 mL
4	0.5 mL	3 mL	0.5 mL	1 mL

Procedure 13.4 Observe the uptake of CO_2 during photosynthesis

1. Fill two test tubes half full with a dilute solution of phenol red provided by your laboratory instructor (fig. 13.8). (Your instructor may have prepared this solution with carbonated water, which is acidic because it has been enriched with CO_2. If this has occurred, the solution will already be yellow, and you should skip to step 3 of this procedure.)
2. Use a straw to gently blow your breath into the phenol red solution. Because excess carbonic acid will lengthen this experiment, stop blowing in the tubes as soon as the color changes to yellow.
3. Add pieces of healthy *Elodea* totaling about 10 cm to one of the tubes. Pour off excess solution above the *Elodea.* The other tube, which is the control, will not include *Elodea.*
4. Cover the tops of the tubes with plastic film or foil to prevent gases from the atmosphere from diffusing into the tubes. Then place both tubes approximately 0.5 m in front of a 100-watt bulb for 30–60 min. What do you think will happen? Write your prediction and a brief explanation here:
5. Observe the tubes about every 10 min.

Question 7

a. What happens to the color of the indicator?

b. What is the reason for the color change?

c. Did the solution in the control tube change color? Why or why not?

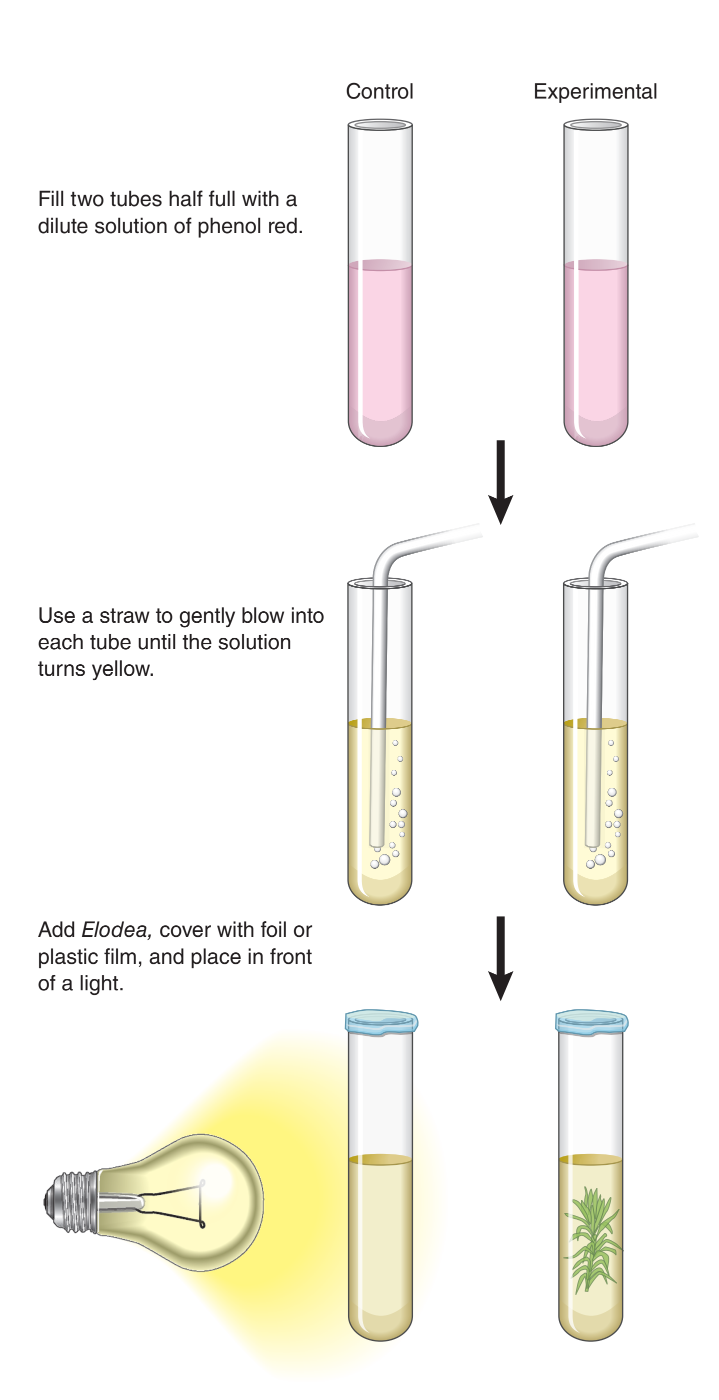


Figure 13.8 Preparation of treatment and control test tubes to determine how photosynthesis affects the pH of a solution.

d. Considering the summary equation for photosynthesis, what is the basis for this change in color?

USE OF LIGHT AND CHLOROPHYLL TO PRODUCE STARCH DURING PHOTOSYNTHESIS

The light-dependent reactions of photosynthesis occur on photosynthetic membranes. In photosynthetic bacteria, these membranes are the cell membrane itself (see fig. 4.2). In plants and algae, photosynthetic membranes are called **thylakoids,** and are located within a special organelle called a **chloroplast** (fig. 13.9; also see fig. 4.8). Thylakoids are stacked to form columns called **grana,** held in place by **lamellae.** A semiliquid **stroma** bathes the interior of the chloroplast and contains the enzymes that catalyze the light-independent reactions of photosynthesis.

Sugars produced by photosynthesis are often stored as starch. Thus, starch production is another indirect measure of photosynthesis. To produce this starch, photosynthesis requires light as an energy source. In the absence of light, sugars and starch are not produced. Photosynthesis also requires chlorophyll to capture light energy. In the absence of chlorophyll, sugars and starch are not produced.

In the following procedures you will detect the presence of starch by staining it with a solution of iodine and observe the requirement of light and chlorophyll for photosynthesis.

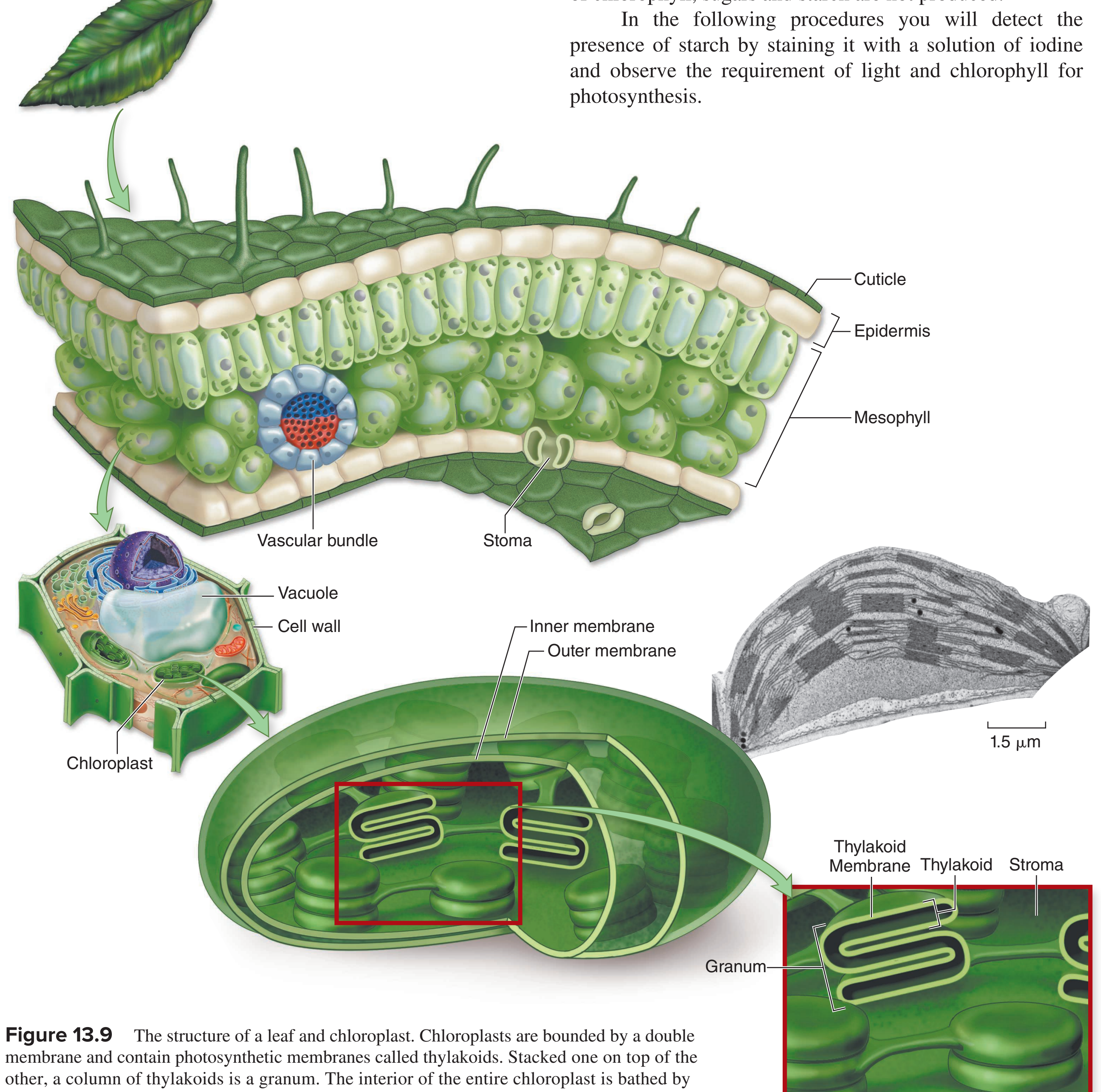


Figure 13.9 The structure of a leaf and chloroplast. Chloroplasts are bounded by a double membrane and contain photosynthetic membranes called thylakoids. Stacked one on top of the other, a column of thylakoids is a granum. The interior of the entire chloroplast is bathed by a semiliquid called the stroma. The openings that enable CO_2 to enter the leaf are stomata (singular, stoma).

Courtesy Dr. Kenneth Miller, Brown University

Procedure 13.5 Stain starch with iodine

1. Place separate drops of water, glucose, and starch solutions on a glass slide.
2. Add a drop of iodine to each and describe your results.

Procedure 13.6 Observe starch production during photosynthesis

1. Remove a leaf from a *Geranium* plant that has been illuminated for several hours.
2. After immersing the leaf in boiling water for 1 min, bleach the pigments from the leaf by boiling the leaf in methanol for 3–5 min. This part of the procedure (i.e., the boiling methanol) must be done in a fume hood. Boiling the leaf will remove pigments so that you can see the color changes of the iodine starch test.



Exercise extreme caution when you heat methanol.

3. Place the leaf in a petri dish containing a small amount of water, and then add five to eight drops of iodine.
4. Observe any color change in the leaf.
5. Record in figure 13.10*a* the color of the leaves after each successive treatment.

Question 8

a. Was starch stored in the leaf? How can you tell?

b. Would you expect leaves to be the primary organ for starch storage in plants? Why or why not?

Procedure 13.7 Observe the requirement of light for photosynthesis

1. Obtain a *Geranium* leaf that has been half or completely covered with metal foil or thick paper for three or four days.
2. Repeat the bleaching and staining steps described in procedure 13.6.
3. Describe and explain any color change in the leaf.
4. Record in figure 13.10*b* the color of the leaves after each successive treatment.

Question 9

Does a leaf produce starch if it has been deprived of light?

Procedure 13.8 Observe the requirement of chlorophyll for photosynthesis

1. Obtain leaves of a variegated *Coleus* plant (fig. 13.11*a*) and a purple-leafed *Coleus* plant (fig. 13.11*b*). Make sketches of their original pigmentation patterns in figure 13.10*c, d.* Indicate which areas are green, red, green/red, and white.
2. Extract the pigments and stain for starch according to procedure 13.6. Boiling the leaf in water will remove the water-soluble pigments such as the red cyanins, and boiling the leaf in alcohol will remove chlorophyll. These pigments must be removed for you to see the color changes of the iodine starch test.
3. Record in figure 13.10*c, d* the color of the leaves after each successive treatment.

Question 10

a. How does the pattern of starch storage relate to the distribution of chlorophyll?

b. Photosynthesis requires chlorophyll (green), but some of the *Coleus* leaves that you tested were purple. How do you explain your results?

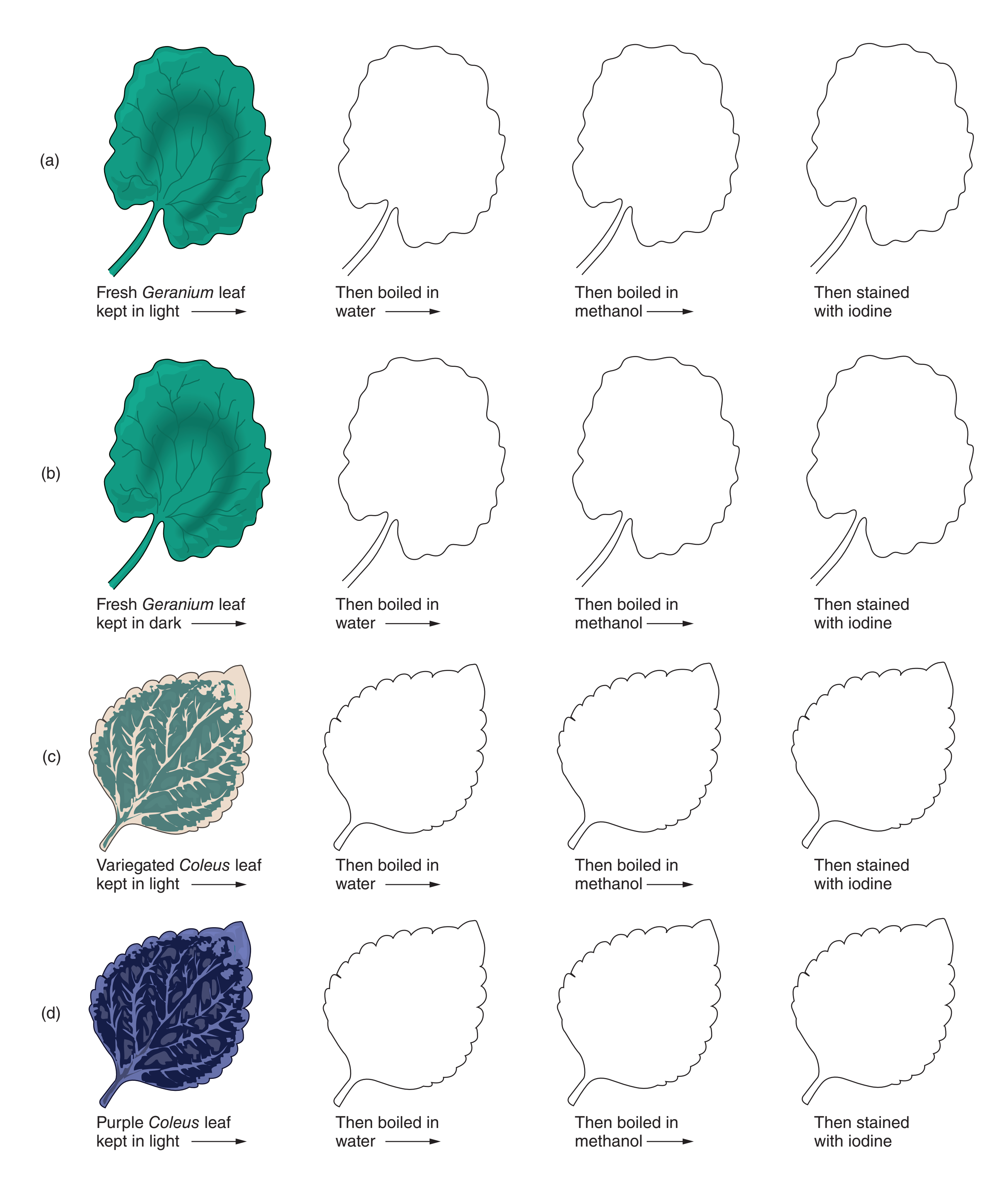
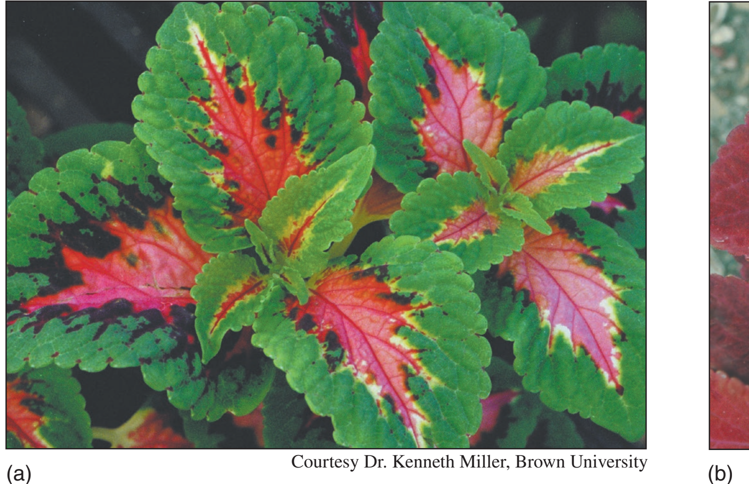


Figure 13.10 The requirement of light and chlorophyll and the production of starch during photosynthesis. Within each diagram, record the color of the leaf following the treatments to indicate (*a*) the production of starch, (*b*) the need for light, and (*c, d*) the need for chlorophyll for photosynthesis. Record your results from the appropriate procedure by writing the resulting color of each treated leaf directly onto the outline of the leaf.



(a) Courtesy Dr. Kenneth Miller, Brown University



(b) © BiologyImaging.com

Figure 13.11 *Coleus* plants. (*a*) Leaves of this variegated plant have green, white, purple, and pink areas resulting from combinations of chlorophylls and anthocyanin (red) pigments. (*b*) Leaves of this purple *Coleus* have the same pigment combination throughout the leaf.

INVESTIGATION

Relative Uptake and Production of CO_2 during Photosynthesis

Observations: Recall from Exercise 12 that aerobic cellular respiration releases CO_2, which can combine with water to form carbonic acid and lower the pH (see procedure 12.3). *Elodea* growing in light respires and photosynthesizes. *Elodea* in darkness only respires. Design an experiment to measure *Elodea's* relative uptake and production of CO_2.

Question: What is the relative uptake versus production of CO_2 during photosynthesis and respiration?

a. Establish a working lab group and obtain Investigation Worksheet 13 from your instructor.

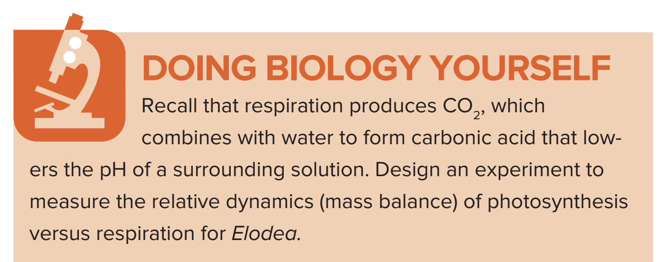
b. Discuss with your group a well-defined question relevant to the preceding observation and question. Record it on Worksheet 13.

c. Translate your question into a testable hypothesis and record it.

d. Review procedure 12.3 that provides a method to quantify CO_2 production. Outline on Worksheet 13 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

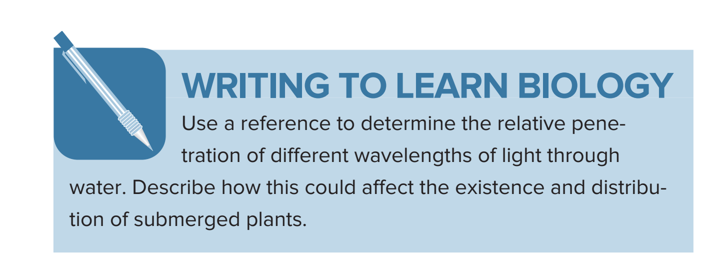
e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.



DOING BIOLOGY YOURSELF

Recall that respiration produces CO_2, which combines with water to form carbonic acid that lowers the pH of a surrounding solution. Design an experiment to measure the relative dynamics (mass balance) of photosynthesis versus respiration for *Elodea*.



WRITING TO LEARN BIOLOGY

Use a reference to determine the relative penetration of different wavelengths of light through water. Describe how this could affect the existence and distribution of submerged plants.

Questions for Further Thought and Study

1. Why does chlorophyll appear green?

2. Is starch produced when a leaf is kept in the dark? Why or why not?

3. What causes leaves to turn from green to yellow and red in autumn?

4. Of what value to plants is starch? Of what value to animals is starch?

5. What is the significance of electron transport in the photochemical (i.e., light-dependent) reactions of photosynthesis?

6. Design an experiment to determine if plants respire. Be sure to explain how you would measure respiration and the controls you would include in the experimental design.



Exercise 14

Mitosis

Replication of Eukaryotic Cells

Learning Objectives

By the end of this exercise you should be able to:

1. Describe events associated with the cell cycle.
2. Describe events associated with mitosis.
3. Distinguish the stages of mitosis on prepared slides of mitotic cells.
4. Stain and examine chromosomes in mitotic cells.
5. Estimate the duration of various stages of mitosis from experimental observations.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Cells grow, have specialized functions, and usually replicate during their life. Although cell enlargement is part of organismal growth, cell replication is also required; this replication allows each cell to grow without becoming too large. All of these activities are part of a repeating set of events called the **cell cycle.** A major feature of the cell cycle is cellular replication, and a major feature of cellular replication is mitosis. **Mitosis** is the replication and division of the nucleus of a eukaryotic cell in preparation for cytokinesis. During mitosis, chromosomes within the cell are replicated by enzymes and then separated into two identical sets—each set is then surrounded by a nuclear membrane. Each of the two new nuclei has a full set of chromosomes containing a copy of all of the genetic information for the organism. Prokaryotic cells lack nuclei and do not undergo mitosis. Instead, they replicate their chromosome and then divide in half during a process called binary fission (described in Exercise 24).

Mitosis is usually associated with **cytokinesis,** the division of the cell and cytoplasm into halves that each contain a nucleus. In some tissues, cytokinesis is delayed or does not occur at all, and the cells are multinucleate. Mitosis and cytokinesis are important because they provide a mechanism for orderly growth of living organisms.

Question 1

Consider the surface-to-volume ratios of large versus small cells. Is it adaptive for cells of a growing organism to remain small? Explain your answer.

THE CELL CYCLE

This exercise emphasizes events associated with mitosis, but mitosis is only part of the cell cycle (fig. 14.1). The remainder of the cycle is called the **interphase** and is subdivided further into **cytokinesis (C), gap 1 (G_1), synthesis (S),** and **gap 2 (G_2)** phases.

The cell cycle begins with the formation of a new cell and ends with replication of that cell. The G_1 phase of the

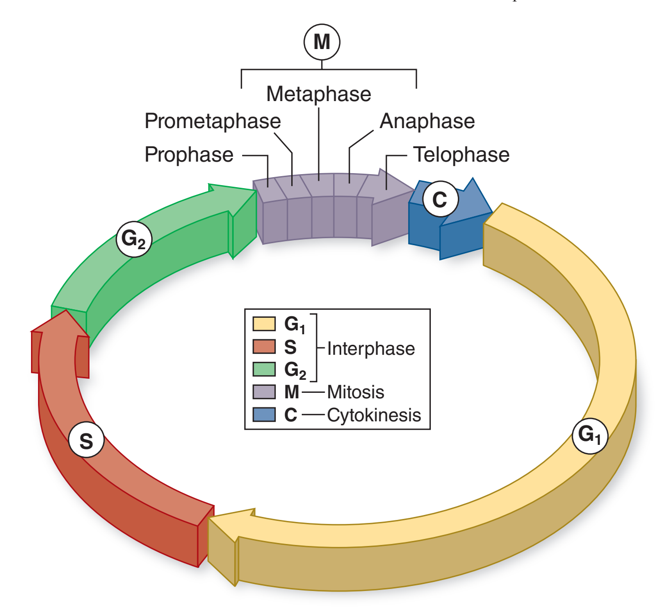


Figure 14.1 The cell cycle is depicted as a circle. The first gap phase, G_1, involves growth and preparation for DNA synthesis. During the S phase, a copy of the genome is synthesized. The second gap phase, G_2, prepares the cell for mitosis. During mitosis, replicated chromosomes are partitioned. Cytokinesis divides the cell into two cells with identical genomes.

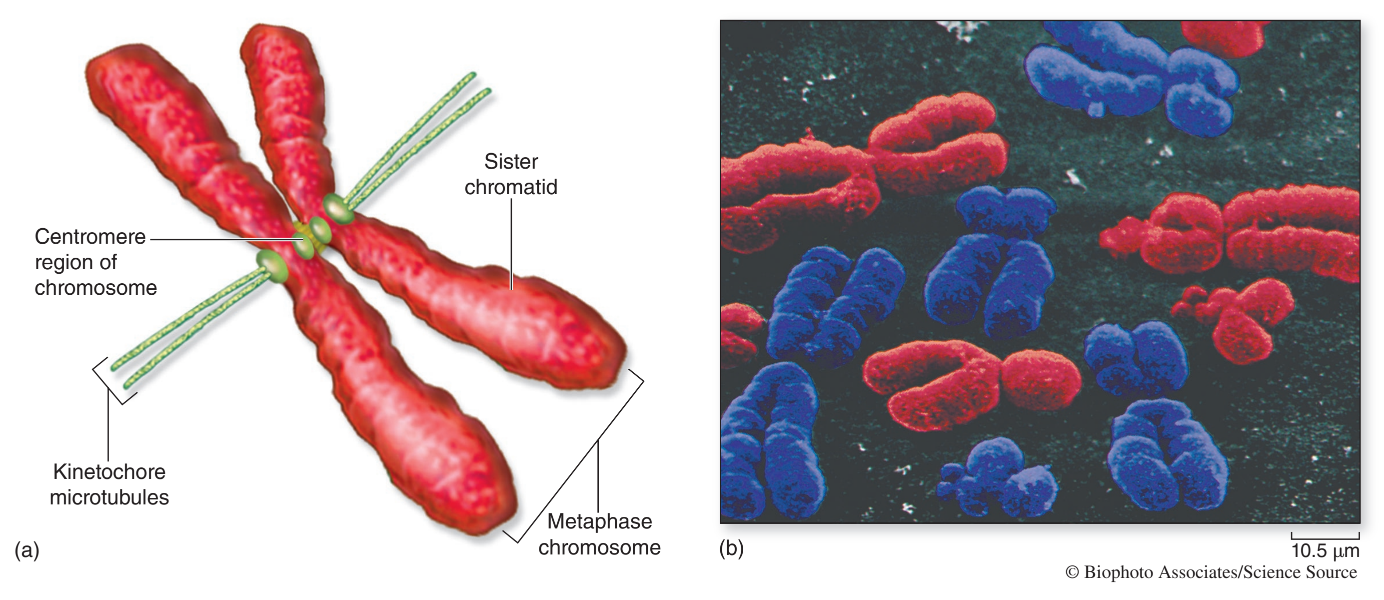


Figure 14.2 Chromosomes. (*a*) In a metaphase chromosome, kinetochore microtubules are anchored to proteins at the centromere. (*b*) This electron micrograph shows how human chromosomes appear during the early stages of nuclear division. Each strand of DNA has already been replicated and condensed to form discrete sister chromatids identical to each other and held together by a centromere.

cell cycle occurs after mitosis and cytokinesis, and is when the majority of cellular activity for the functions of the cell occurs. Many cell-specific proteins and other molecules are produced for the metabolism of the cell during G_1. During the S phase, the DNA composing the chromosomes is duplicated. At the end of the S phase each chromosome consists of an identical pair of chromosomal DNA strands, called sister **chromatids,** attached at a **centromere** (fig. 14.2). During the G_2 phase, molecules and structures necessary for mitosis are synthesized.

Mitosis (M phase) usually lasts for less than 10% of the time of the cell cycle, which usually lasts 10 to 30 h.

(a) Interphase

Chromosomes
Nuclear membrane
Two centrosomes, each with centriole pairs

1 Chromosomes have already replicated during interphase.

(b) Prophase

Sister chromatids

2 Sister chromatids condense and spindle starts to form. Nuclear membrane begins to dissociate into vesicles.

(c) Prometaphase

Mitotic spindle
Spindle pole
Kinetochore microtubule

3 Nuclear membrane has completely dissociated into vesicles and the spindle is fully formed. Sister chromatids attach to spindle via kinetochore microtubules.

Figure 14.3 Interphase and the stages of mitosis in an animal cell. The cleavage furrow signifying cytokinesis may first appear during anaphase or more typically during telophase.

Actively dividing cells such as those in rapidly growing tissues may spend more than 10% of their time in mitosis, whereas static cells such as bone cells or neurons may rarely enter M phase. Cytokinesis may begin during mitosis but is highly variable in length and timing. Tissues such as striated muscle fibers, and some algal filaments, may undergo mitosis without cytokinesis and produce multinucleate cells.

Question 2

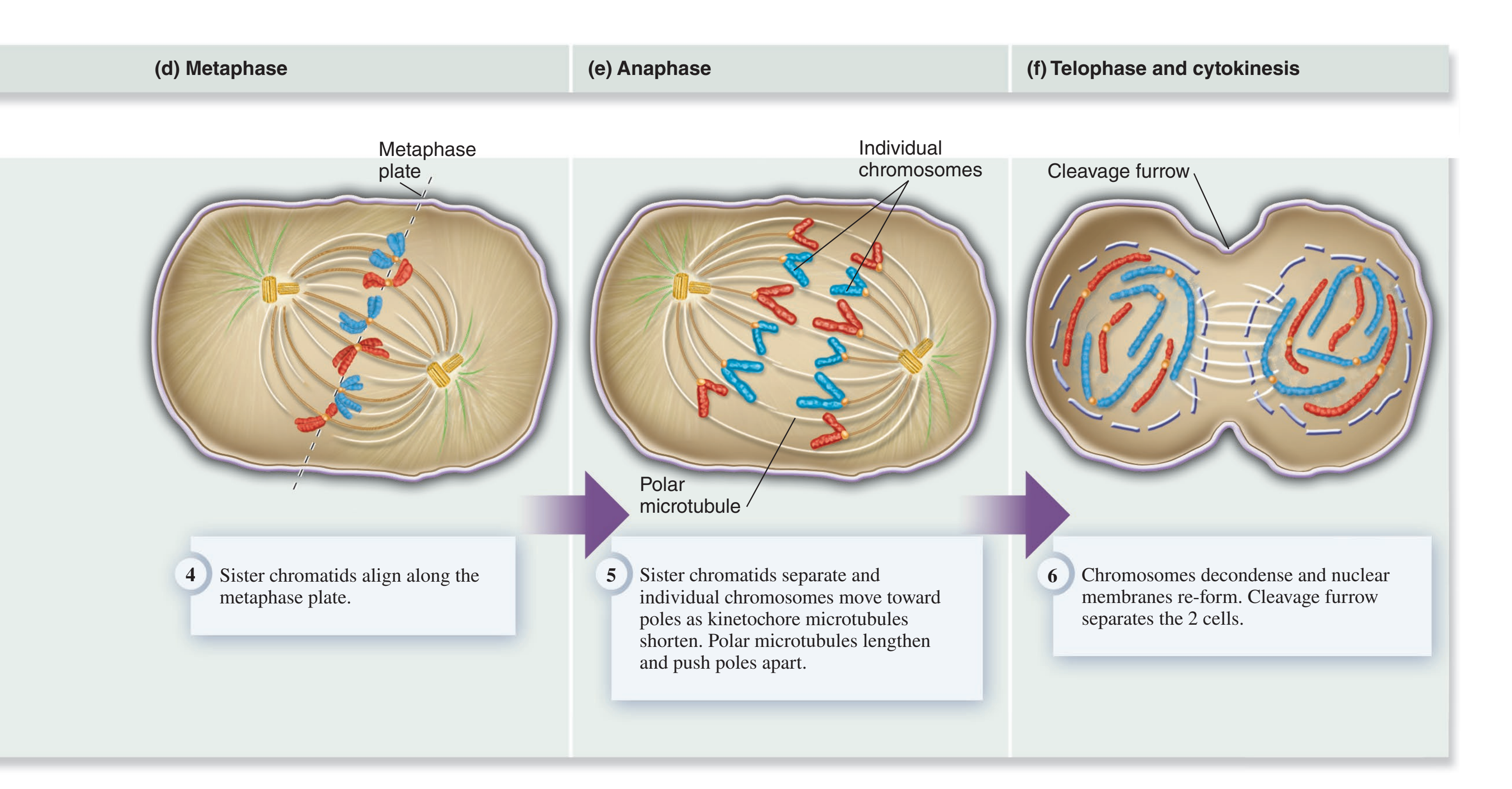
a. Mitosis and cytokinesis are often referred to collectively as "cellular division." Why are they more accurately called cellular replication?

b. Does the cell cycle have a beginning and an end? Explain.

STAGES AND EVENTS OF MITOSIS

Mitosis (1) separates the genetic material duplicated during interphase into two identical sets of chromosomes, and (2) reconstitutes a nucleus to house each set. As a result, mitosis produces two identical nuclei from one. In animals, mitosis occurs in body (i.e., non-sex) cells.

Mitosis is traditionally divided into five stages: **prophase, prometaphase, metaphase, anaphase,** and **telophase** (fig. 14.3). The actual events of mitosis are not discrete but occur in a continuous sequence; separation of mitosis into five stages is merely convenient for our discussion and organization. During these stages, important cellular structures are synthesized and perform the mechanics of mitosis (fig. 14.3). For example, in animal cells, two microtubule-organizing centers called **centrosomes** contain cylinders of microtubules called **centrioles,** which replicate at the onset of mitosis. The pairs of centrioles move apart and form an axis of proteinaceous microtubules between them called **polar microtubules.** The centrioles continue to move apart until they reach opposite poles of the cell and have a bridge of microtubules called the **mitotic spindle** (or spindle apparatus) extending between them (fig. 14.4). **Kinetochore microtubules** (fig. 14.2) attach to each chromosome's **kinetochore,** which is a complex of proteins that binds to the centromere. At the poles of the cell, the centrioles radiate an array of microtubules outward in addition to the spindle apparatus. These microtubules brace the centrioles against



Cancer — A Corrupt Cell Cycle

Cancer is unrestrained cell proliferation caused by damage to genes that regulate the cell division cycle. Cancer produces a cluster of cells called a *tumor* that constantly expands. Tumors from cells in connective tissue, bone, or muscle are known as **sarcomas,** while those from epithelial tissue, such as skin, are called **carcinomas** (fig.14.A). In the United States, the four deadliest human cancers (about 55% of all cancer deaths) are lung cancer, colon cancer, breast cancer, and leukemia/lymphomas. Recent work has identified one of the culprits in cancer. Officially dubbed p53, this gene plays a key role in the G_1 check-point of cell division (fig. 14.B). The gene's product—the p53 protein—monitors the integrity of DNA, checking that it is undamaged. If the p53 protein detects damaged DNA, it halts cell division and stimulates enzymes to repair the damage. After repair, p53 allows cell division to continue. If the DNA damage is irreparable, then the p53 directs the cell to kill itself.

The p53 gene prevents the development of many mutated cells and is therefore a **tumor-suppressor gene.** Researchers have found that p53 is absent or damaged in the majority of cancerous cells they have examined. It is precisely because p53 is nonfunctional that cancer cells are able to repeatedly undergo cell division without being halted at the G_1 phase of the cell cycle.

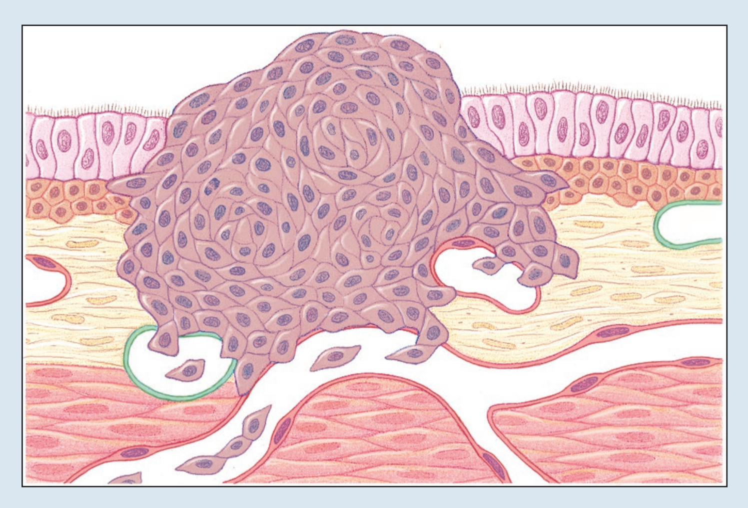


Figure 14.A Portrait of a cancer. This carcinoma is developing from epithelial cells that line the interior of a human lung. As the mass of cells grows, it invades the surrounding tissues, eventually penetrating lymphatic and blood vessels, both of which are plentiful in the lung. These vessels carry metastatic cancer cells throughout the body, where they lodge and grow, forming new masses of cancerous tissue.

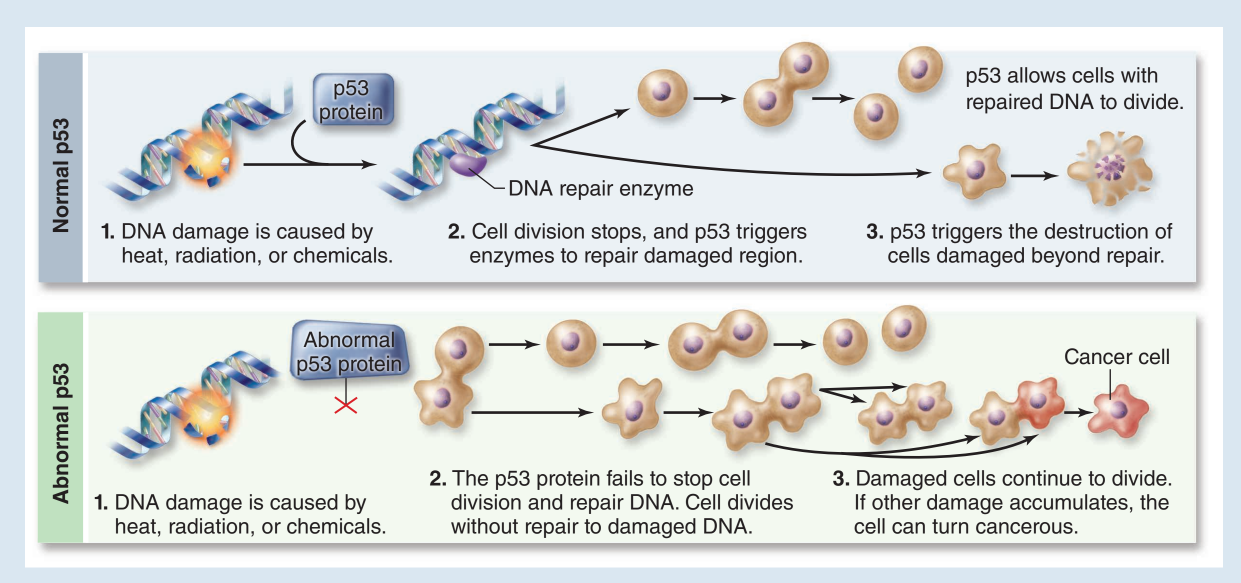


Figure 14.B Cell division, cancer, and p53 protein. Normal p53 protein monitors DNA, destroying cells that have irreparable damage to their DNA. Abnormal p53 protein fails to stop cell division or repair DNA. As damaged cells proliferate, cancer develops.

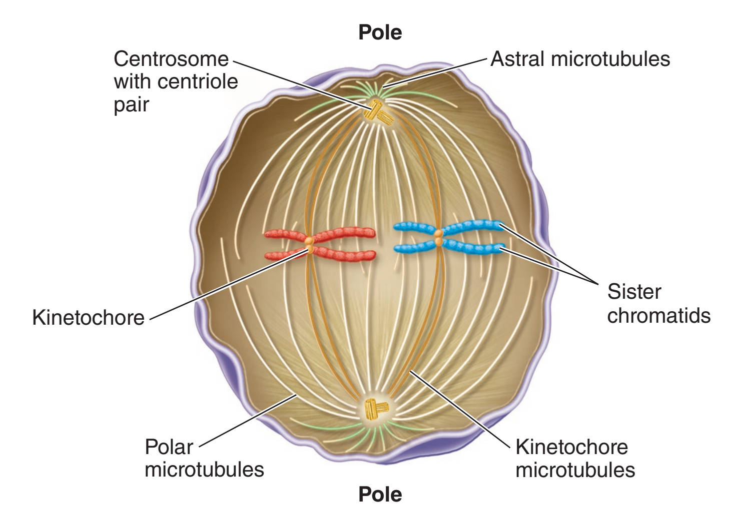


Figure 14.4 The structure of the mitotic spindle. The mitotic spindle is formed by the centrosomes from three types of microtubules. The astral microtubules emanate away from the region between the poles. The polar microtubules project into the region between the two poles. The kinetochore microtubules are attached to the kinetochores of sister chromatids.

the cell membrane. This arrangement of microtubules radiating from a centriole is called an **aster** with **astral microtubules.** Plant cells lack centrioles and asters, but spindle fibers still form between opposite poles of the cell.

Interestingly, animal cells deprived of centrioles will still form a spindle apparatus. Chromosomes will eventually distribute themselves on the spindle apparatus and are moved and separated to opposite poles. The distribution of chromosomes will also occur if the cell is haploid (i.e., has a single set of chromosomes). The vegetative cells of many organisms such as fungi are haploid rather than diploid (have a double set of chromosomes). However, the steps of mitosis are the same as for diploid cells.



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 14.1 Describe the specific events of mitosis

Before your lab meeting, review in your textbook the events associated with each stage of mitosis in addition to the preparatory stage, interphase. List these events in table 14.1. Some events and structures occur only in plant cells and some occur only in animal cells. Mark these events in your

Table 14.1

Events of Mitosis and Interphase
Interphase Although interphase is not part of nuclear replication, understanding its events is essential to understanding mitosis.
Prophase
Prometaphase
Metaphase
Anaphase
Telophase

list with an asterisk. This list can serve as an excellent study guide, so be as complete as possible. One event for each stage is provided in figure 14.3.

Question 3

a. If a nucleus has eight chromosomes during interphase, how many chromosomes does it have during metaphase?

b. How many does it have after mitosis is complete?

Understanding the movements of chromosomes is crucial to understanding mitosis. You can simulate these movements easily with chromosome models made of pipe cleaners or popsicle sticks. This is a simple procedure but a valuable one. It will be especially helpful when you are comparing the events of mitosis to the events of meiosis, which you will simulate in the next exercise.

Procedure 14.2 **Simulate chromosomal replication and movement during mitosis**

1. Examine the materials to be used as chromosome models provided by your instructor.
2. Identify the differences in chromosomes represented by various colors, lengths, or shapes of materials. Also identify materials representing centromeres.
3. Place a sheet of notebook paper on your lab table to use in representing the boundaries of the mitotic cell.
4. Assemble the chromosomes needed to represent nuclear material in a cell of a diploid organism with a total of six chromosomes. Place the chromosomes in the cell.
5. Arrange the chromosomes to depict the position and status of chromosomes during interphase G_1. (During G_1 the chromosomes are usually not condensed, as the chromosome models imply, but the models are an adequate representation.)
6. Depict the status of chromosomes after completing interphase S. Use additional "nuclear material" if needed.
7. Move the chromosome models appropriately to depict prophase.
8. Move the chromosome models appropriately to depict metaphase.
9. Move the chromosome models appropriately to depict anaphase.
10. Move the chromosome models appropriately to depict telophase.
11. Draw the results of cytokinesis and the re-formation of nuclear membranes.
12. Chromosomal events occur as a continuous process of movements rather than in distinct steps. Therefore, repeat steps 4–11 as a continuous process and ask your instructor to verify your simulation.

MITOSIS IN ANIMAL CELLS

The most distinctive features of cellular replication in animal cells are the formation of asters with centrioles at their center (discussed earlier) and cytokinesis. Cytokinesis includes formation of a **cleavage furrow** that begins on the periphery of the cell, pinches inward, and eventually divides the cytoplasm into two cells (fig. 14.5). Cells of a whitefish blastula provide good examples of the stages of mitosis and cytokinesis. Whitefish are commonly cultured fish whose eggs and early developmental stages undergo rapid cell divisions (as do all embryonic cells). A blastula is an early embryonic stage of a vertebrate and consists of a sphere of 25–100 cells with a high frequency of different mitotic stages. Exercise 50 (Embryology) details the formation of a blastula during embryonic development.

Procedure 14.3 **Observe and describe mitosis in animal cells**

1. Obtain a prepared slide of a cross section through the blastula of a whitefish.
2. Examine the cells first on low (10×) then high (40× or 100×) magnification. Some of the cells contain condensed and stained chromosomes.
3. Refer to figure 14.3 for a summary of the stages of mitosis. Identify examples of each stage on your

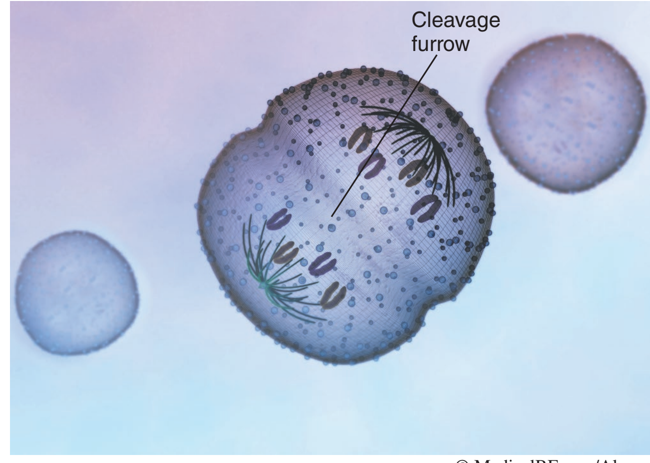


Figure 14.5 Cytokinesis in an animal cell. Cytokinesis, the physical division of the cell's cytoplasm, usually occurs after nuclear replication is complete. A cleavage furrow is forming around this dividing sea urchin egg.

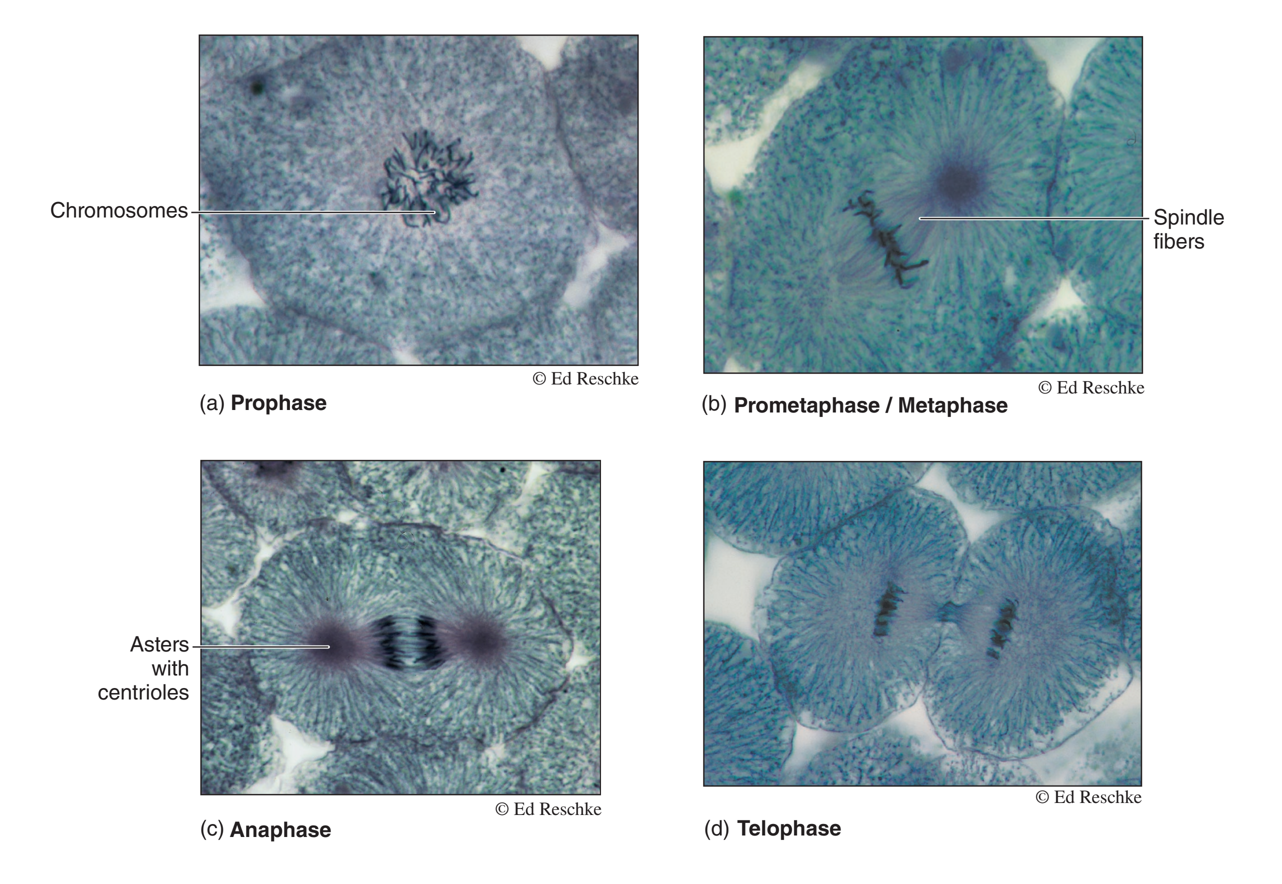


Figure 14.6 Stages of mitosis in cells of a whitefish embryonic blastula (400×). Prometaphase and metaphase may not always be distinguishable by light microscopy.

prepared slide (fig. 14.6). Verify these stages with your lab partner or teaching assistant.

4. Also identify cells that you believe are between stages.
5. Examine the whitefish cells for signs of cytokinesis.
6. Prepared cross-sections of cells show only two dimensions, but mitosis is a three-dimensional process. In the following space, draw two cells in metaphase: one in which the cross section is parallel to the axis of the spindle apparatus and one in which the cross section is perpendicular to the spindle apparatus.

Question 4

a. Why would we choose an embryonic mass of cells for procedure 14.3 in which to study the stages of mitosis?

b. Which stage of mitosis most often is associated with the beginning of cytokinesis?

MITOSIS IN PLANT CELLS

Our model to study cellular replication in plants is the root tip of *Allium* (onion). Root tips of plants contain **meristems,** which are localized areas of rapid cell division due to active growth at the root tips. In plant cells, cytokinesis includes formation of a partition called a **cell plate** perpendicular to the axis of the spindle apparatus. The cell plate forms in the middle of the cell and grows out to the periphery. It will separate the two new cells.

Interestingly, the formation of the spindle apparatus and other microtubule systems in plant and fungal cells is organized by centrosomes, as in animal cells. But plant and fungal cells have no centrioles within the centrosomes. Thus, the function and necessity of centrioles remain somewhat of a mystery.

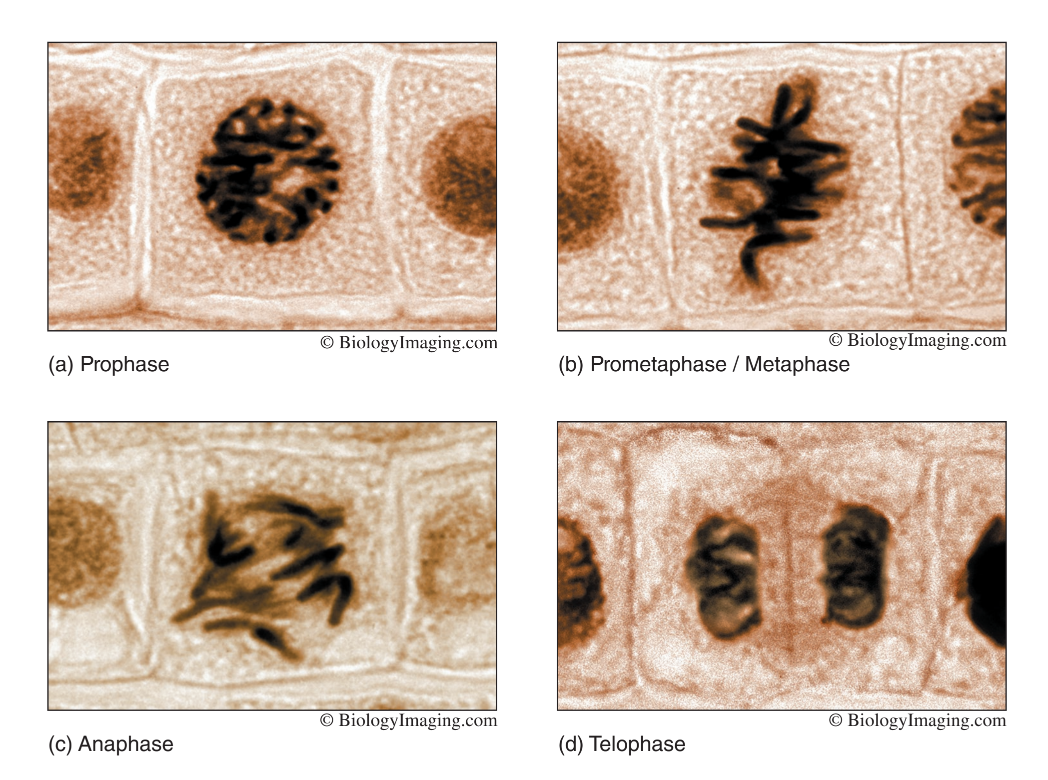


Figure 14.7 Stages of mitosis in a plant cell (1000×). The dark structures are chromosomes.

Procedure 14.4 Observe and diagram mitosis in plant cells

1. Examine a prepared slide of a longitudinal section through an onion root tip.
2. Search for examples of all stages of mitosis (fig. 14.7). Notice that most cells are in some part of interphase. Prometaphase may be difficult to distinguish from metaphase.
3. Search for signs of cell plate formation.
4. In figure 14.8, diagram a plant cell with a diploid number of three pairs of chromosomes in each of the stages of mitosis. **Diploid** refers to a nucleus with two of each type of chromosome. Be sure to label the cell wall and cell plate.
5. Prepared cross sections of cells show only two dimensions, but mitosis is a three-dimensional process. In the following space draw two cells in metaphase: one in which the plane of section is parallel to the axis of the

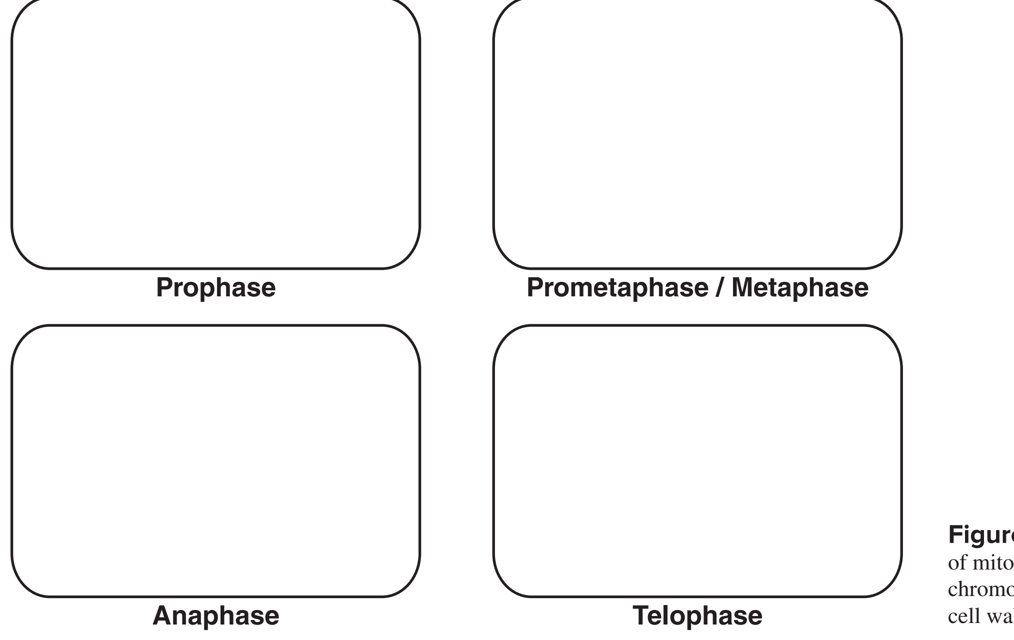


Figure 14.8 Diagram the stages of mitosis in a plant cell with six chromosomes. The outlines represent the cell walls of each of four cells.

INVESTIGATION

The Time Elapsed during the Various Stages of Mitosis

Observations: The cell cycle of actively dividing cells of root tips of *Allium* is approximately 24 h long. The phases of mitosis usually occupy only a small portion of that time.

Question: How long does mitosis take?

a. Establish a working lab group and obtain Investigation Worksheet 14 from your instructor.

b. Discuss with your group a well-defined question relevant to the preceding observation and question. Record it on Worksheet 14.

c. Translate your question into a testable hypothesis and record it.

d. Each prepared slide of a root tip of *Allium* reveals a snapshot in time of all stages of mitosis.

e. The relative abundance of cells in a phase of mitosis is directly proportional to the length of time for that phase.

f. Outline on Worksheet 14 your experimental design and supplies needed to test your hypothesis. The table below offers insight to a reasonable design. Ask your instructor to review your proposed investigation.

g. Conduct your procedures, record your data, answer your question, and make relevant comments.

h. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Stage of Mitosis	Predicted Duration (hours)	Number of Cells in Each Stage	Total Number of Cells in Each Stage	Class Total Number for Each Stage	Calculated Duration (hours)
Interphase	__________	____ ____ ____	__________	__________	__________
Prophase	__________	____ ____ ____	__________	__________	__________
Prometaphase/ Metaphase	__________	____ ____ ____	__________	__________	__________
Anaphase	__________	____ ____ ____	__________	__________	__________
Telophase	__________	____ ____ ____	__________	__________	__________
Totals	__________	____ ____ ____	__________	__________	__________

spindle apparatus and one in which the cross section is perpendicular to the spindle apparatus.

Question 5

a. What region of a root has the most mitotic activity?

b. How does cytokinesis differ in plant versus animal cells?

c. Why is pinching of the cytoplasm inadequate for cytokinesis in plant cells?

d. What is a cell plate, and in what stage of mitosis does it form?

e. Locate a plant cell in late telophase. What is the volume of the two new cells relative to a mature cell?

PREPARING AND STAINING CHROMOSOMES

Your instructor has prepared some living onion root tips for you to process further and use to observe the stages of mitosis.

Procedure 14.5 **Stain chromosomes**

1. Obtain an onion root tip and place it in a small vial with Schiff's reagent for 30 min. Handle Schiff's reagent carefully because it is a colorless liquid that becomes bright red after reaction.
   Keep the vial in the dark and at room temperature until the root tip becomes purple. Your instructor may have already stained some root tips for you.
2. Place the root tip in a drop of 45% acetic acid on a slide and cut away and remove all of the root tissue except the terminal 1 mm of the tip.



Acetic acid is corrosive. Do not spill it.

3. Crush the root tip with a blunt probe and cover the tissue with a coverslip.
4. Smash the tissue by pressing on the coverslip with the eraser of your pencil. Your instructor will demonstrate this procedure.
5. Scan your preparation at low magnification to locate stained chromosomes. Then switch to high magnification and locate formations of chromosomes that indicate each of the stages of mitosis.
6. Add a drop of acetic acid to the edge of the coverslip to avoid desiccation.
7. Locate as many stages of mitosis as you can. Be sure to look at preparations done by other students.

Questions for Further Thought and Study

1. Interphase has sometimes been called a "resting stage." Why is this inaccurate?

2. Most general functions of a cell occur during G_1 of interphase. What events that occur during other phases of the cell cycle might inhibit general metabolism?

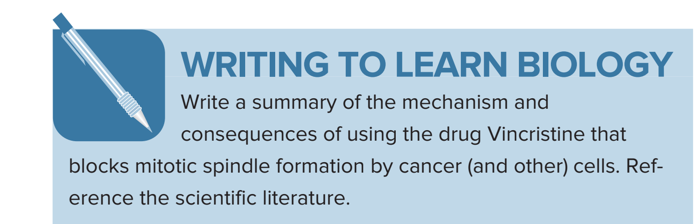
3. Read in your textbook about prokaryotic cellular replication; list the fundamental cellular/structural differences between it and eukaryotic cellular replication. What is the basis for these differences?

4. Some specialized cells such as neurons and red blood cells lose their ability to replicate when they mature. Which phase of the cell cycle do you suspect is terminal for these cells? Why?

5. Find a concise definition of "cancer." How might methods to treat cancer relate to what you learned in this lab exercise?

WRITING TO LEARN BIOLOGY

Refer to your textbook to review the properties of a chemical called colchicine. Describe how colchicine affects dividing cells. What is the mechanism of this effect? How might colchicine be used as a tool in scientific research or medicine?



WRITING TO LEARN BIOLOGY

Write a summary of the mechanism and consequences of using the drug Vincristine that blocks mitotic spindle formation by cancer (and other) cells. Reference the scientific literature.

Meiosis

Reduction Division and Gametogenesis

Learning Objectives

By the end of this exercise you should be able to:

1. Describe the events of meiosis.
2. Compare and contrast meiosis and mitosis.
3. List the most significant events of meiosis.
4. Explain the relevance of meiosis to sexual reproduction and evolutionary change.
5. Explain the relationship of meiosis and gametogenesis.
6. Describe the events of spermatogenesis and oogenesis.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Sex is one of the most experienced and scrutinized processes of life. Biologists know that the significance of sex, and meiosis in particular, is the recombination of a parent's genes and the packaging of these genes as a gamete. During sexual reproduction a gamete and its genes are combined with another parent's gamete and genes to endow the new offspring with new genetic combinations.

Chromosomes in typical, eukaryotic nuclei occur in pairs; that is, the nuclei are **diploid** (2*n*). The two chromosomes of a pair are called **homologous chromosomes,** and each homologue of a pair has the same sites, or **loci,** for the same genes, although the homologues may carry different alleles at homologous loci. A nucleus, such as that in a gamete, with only one chromosome of each homologous pair is **haploid** (*n*).

Meiosis produces haploid daughter nuclei and is sometimes called "reduction division." Reducing the number of chromosomes in the nucleus of a gamete to only one of each pair is important because such a haploid nucleus can fuse with another haploid nucleus during sexual reproduction and restore the original, diploid number of chromosomes to the new individual (fig. 15.1).

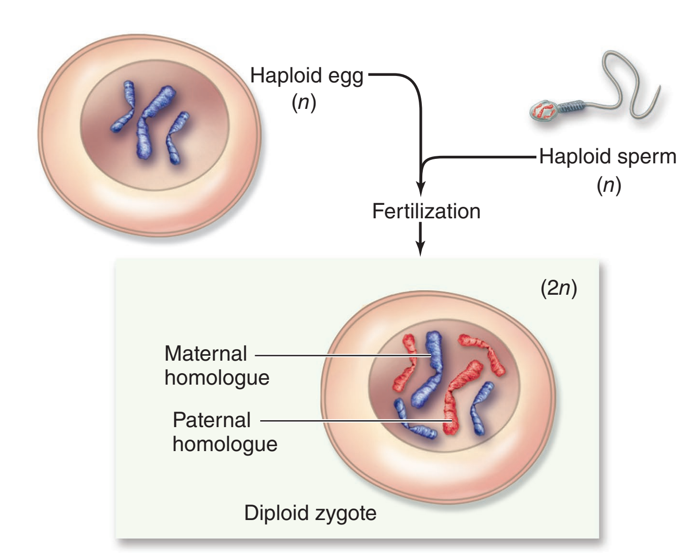


Figure 15.1 In animals, meiosis produces eggs and sperm, both of which are haploid (*n*). Fusion of an egg and sperm during fertilization produces a zygote, which is diploid (2*n*).

Question 1

a. Why would shuffling genetic material to produce new combinations of characteristics be advantageous to a species?

b. When would it be deleterious?

Meiosis, like mitosis, is preceded by the replication of each chromosome to form two sister chromatids attached at a centromere (fig. 15.2). However, two events that do *not* occur in mitosis include final reduction of the chromosome number by half and production of new genetic combinations. Meiosis reduces the chromosome number during *two* rounds of chromosome separation called **meiosis I** and **II.** Thus, the genetic material is replicated once just before meiosis but divided twice during meiosis. This allocates half the

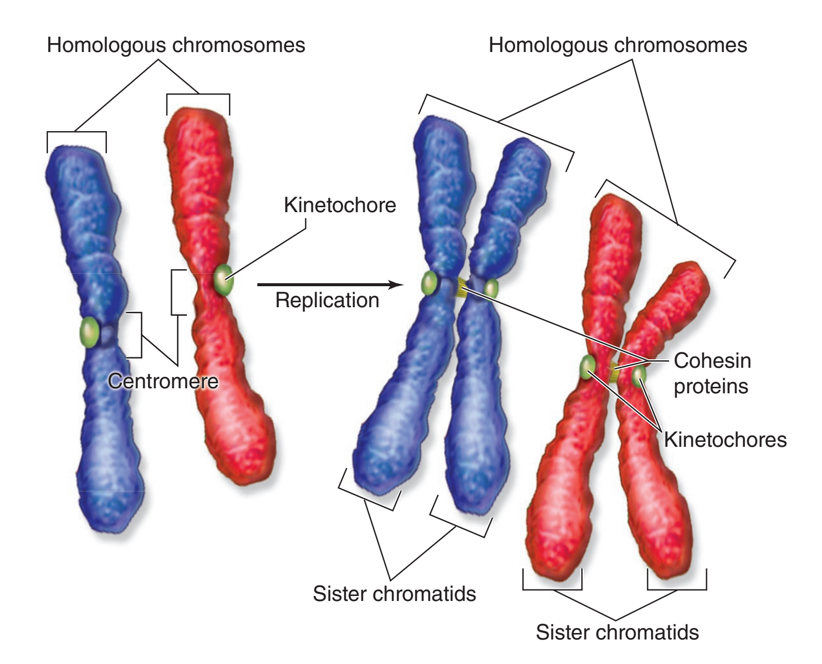


Figure 15.2 The difference between homologous chromosomes and sister chromatids. Homologous chromosomes are the maternal and paternal copies of the same chromosome—say, chromosome number 16. Sister chromatids are the two replicas of a single chromosome held together at their centromeres by cohesin proteins after DNA replication. The kinetochore is composed of proteins found at the centromere that attach to microtubules during mitosis.

original number of chromosomes (one of each original pair) to each daughter cell; that is, the nuclei are haploid.

To produce new genetic combinations each chromosome (composed of two sister chromatids) initially pairs along its length with its homologue to form a **bivalent** (fig. 15.3). This pairing of homologous chromosomes is called **synapsis,** and the four chromatids exchange homologous segments of genetic material called **alleles.** Alleles are alternate states of a gene, such as a Type A allele, Type B allele, or Type O allele, which together determine a person's blood type. This exchange of genetic material among chromatids is called **crossing-over** and produces new genetic combinations. During crossing-over there is no gain or loss of genetic material. But afterward, each chromatid of the chromosomes contains different segments (alleles) that it exchanged with other chromatids. The temporary joints of two chromatids at a point of genetic exchange are called **chiasmata** (fig. 15.3).

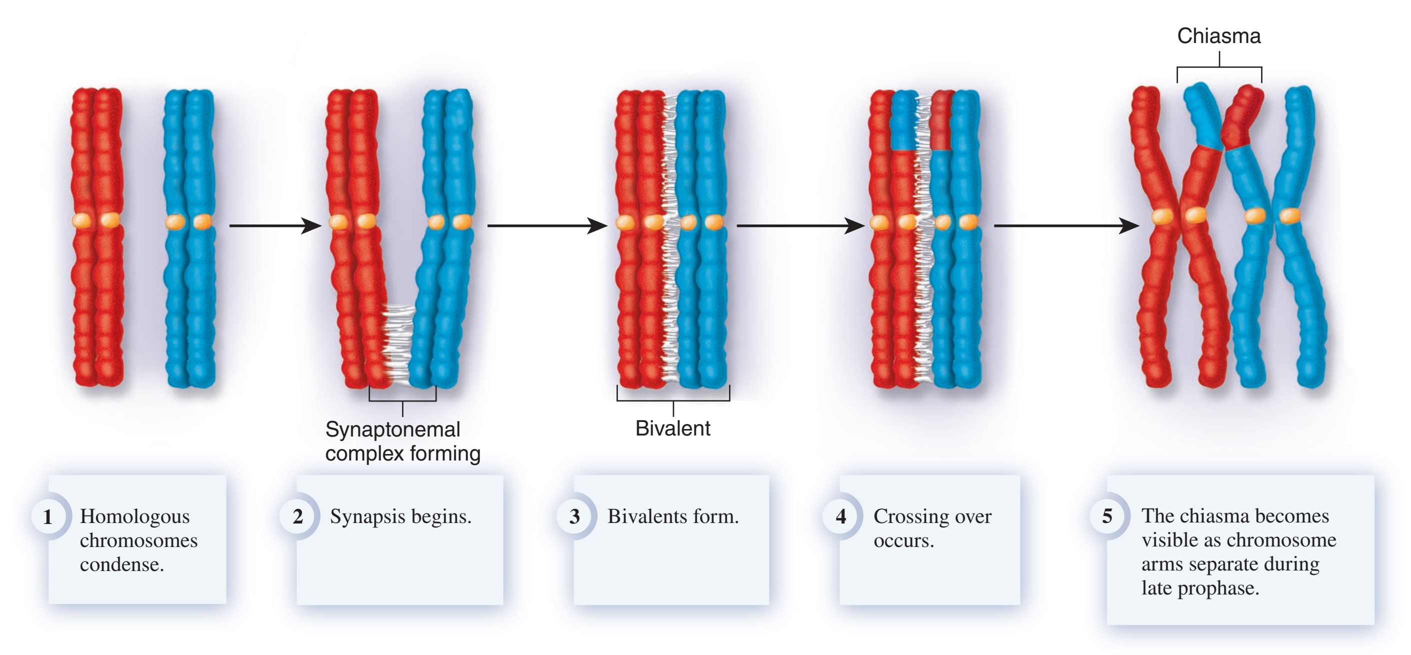


Figure 15.3 Formation of a bivalent and crossing-over during meiosis I. At the beginning of meiosis, homologous chromosomes pair with each other to form a bivalent, usually with a synaptonemal complex between them. Crossing-over then occurs between homologous chromatids within the bivalent. During this process, homologues exchange segments of chromosomes.

Question 2

a. Synapsis occurs after chromosomal DNA has replicated. How many chromatids are involved in crossing-over of a homologous pair of chromosomes?

b. Suppose synapsis occurred between two homologous chromosomes, and one had alleles for blue eyes and brown hair where the other had alleles for green eyes and blonde hair. How many different combinations of these alleles would be possible?

STAGES AND EVENTS OF MEIOSIS

Although meiosis is a continuous process, we can study it more easily by dividing it into stages just as we did for mitosis. Meiosis and mitosis are similar, and their corresponding stages of prophase, prometaphase, metaphase, anaphase, and telophase have much in common. However, meiosis takes longer than mitosis because meiosis involves two divisions instead of one. These two reductions are called meiosis I and meiosis II. Homologous chromosomes are separated at the end of meiosis I, and chromatids composing each chromosome are separated during meiosis II. Each reduction involves the events of prophase, prometaphase, metaphase, anaphase, and telophase (fig. 15.4).

Before your lab meeting, review in your textbook the events associated with each stage of meiosis, including the preparatory stage, interphase. List these events in table 15.1. This list can serve as an excellent study guide, so be as complete as possible. Ask your instructor to check for errors. One or two events for each stage are provided in figure 15.4.

Premeiotic Interphase

Meiosis I is preceded by an interphase similar to the G_1, S, and G_2 of mitotic interphase, including replication of the chromosomes. Each chromosome is replicated.

Compare the outline in table 15.1 with your outline in table 14.1 on mitosis.

Question 3

a. If a nucleus has eight chromosomes when it begins meiosis, how many chromosomes does it have after telophase I? Telophase II?

b. What are the major differences between the events of meiosis and mitosis?

c. What are some minor differences, and why do you consider them minor?

Understanding the movements of chromosomes is crucial to understanding meiosis. You can simulate these movements easily with chromosome models made of pipe cleaners or popsicle sticks. This simple but valuable exercise is especially instructive if you compare your simulation of meiosis with your simulation of mitosis from the previous exercise. There are important differences between them.

Procedure 15.1 **Simulate chromosomal replication and movement during meiosis**

1. Examine the materials for chromosome models provided by your instructor.
2. Identify the differences in chromosomes represented by various colors, lengths, or shapes of the materials. Also identify materials representing centromeres.
3. Place a sheet of notebook paper on your lab table. Use it to represent the boundaries of the meiotic cell.
4. Assemble the chromosomes needed to represent the nuclear material in a cell of a diploid organism with a total of six chromosomes (three homologous pairs). Place the chromosomes in the cell.
5. Arrange the chromosomes to depict the position and status of chromosomes during interphase G_1. (During G_1 the chromosomes are usually not condensed as the chromosome models imply; nevertheless, the models are an adequate representation.)
6. Depict the chromosomes after completing interphase S. Use additional "nuclear material" if needed.
7. Depict the chromosomes during prophase I, metaphase I, anaphase I, and telophase I.
8. The interval between meiosis I and meiosis II is called **interkinesis.** Draw the results of cytokinesis, which occurs at this stage in some organisms.
9. Depict the status of chromosomes during prophase II for both daughter nuclei. Repeat this for metaphase II, anaphase II, and telophase II.
10. Draw the results of cytokinesis and the re-formation of nuclear membranes.

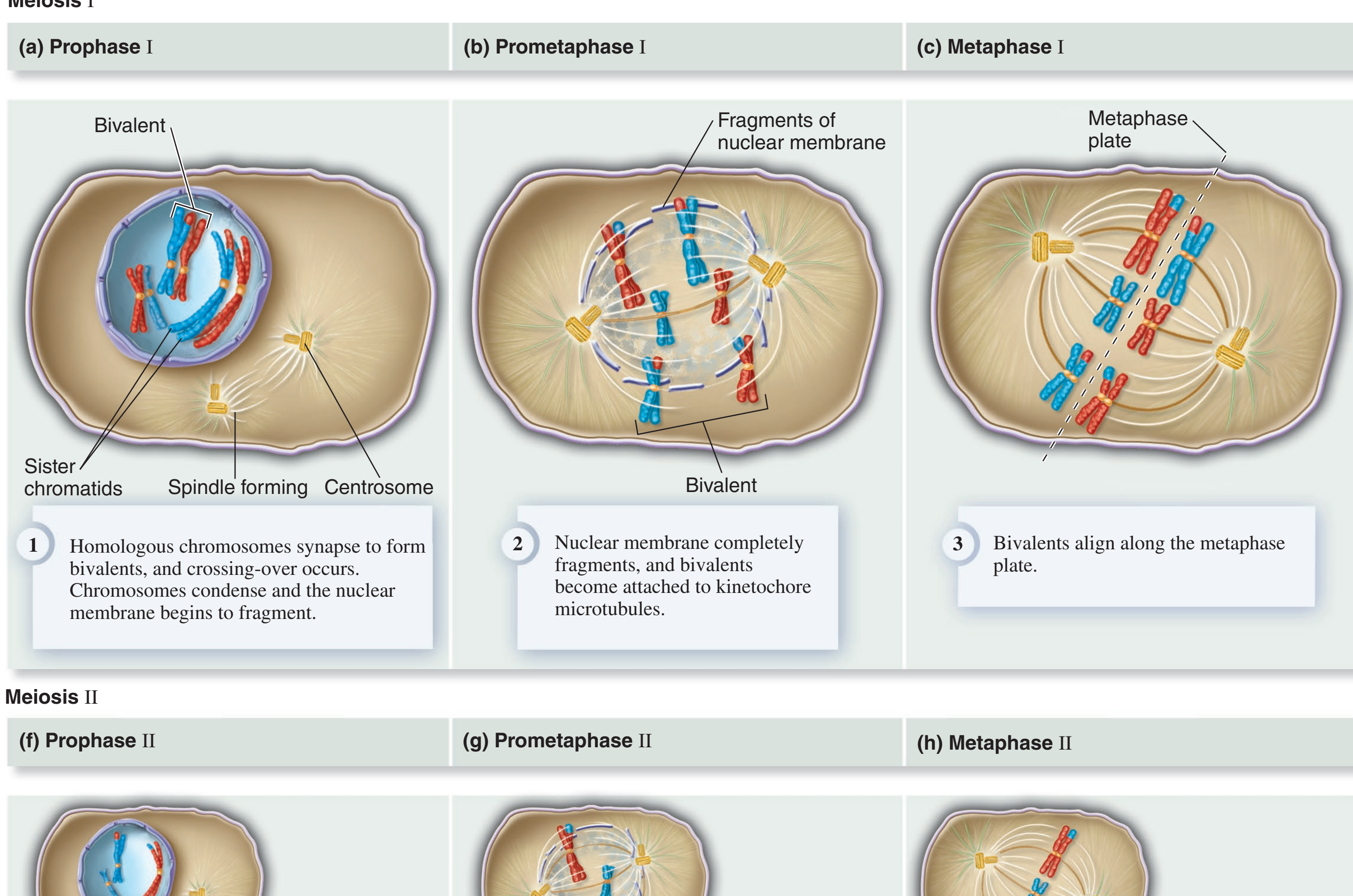


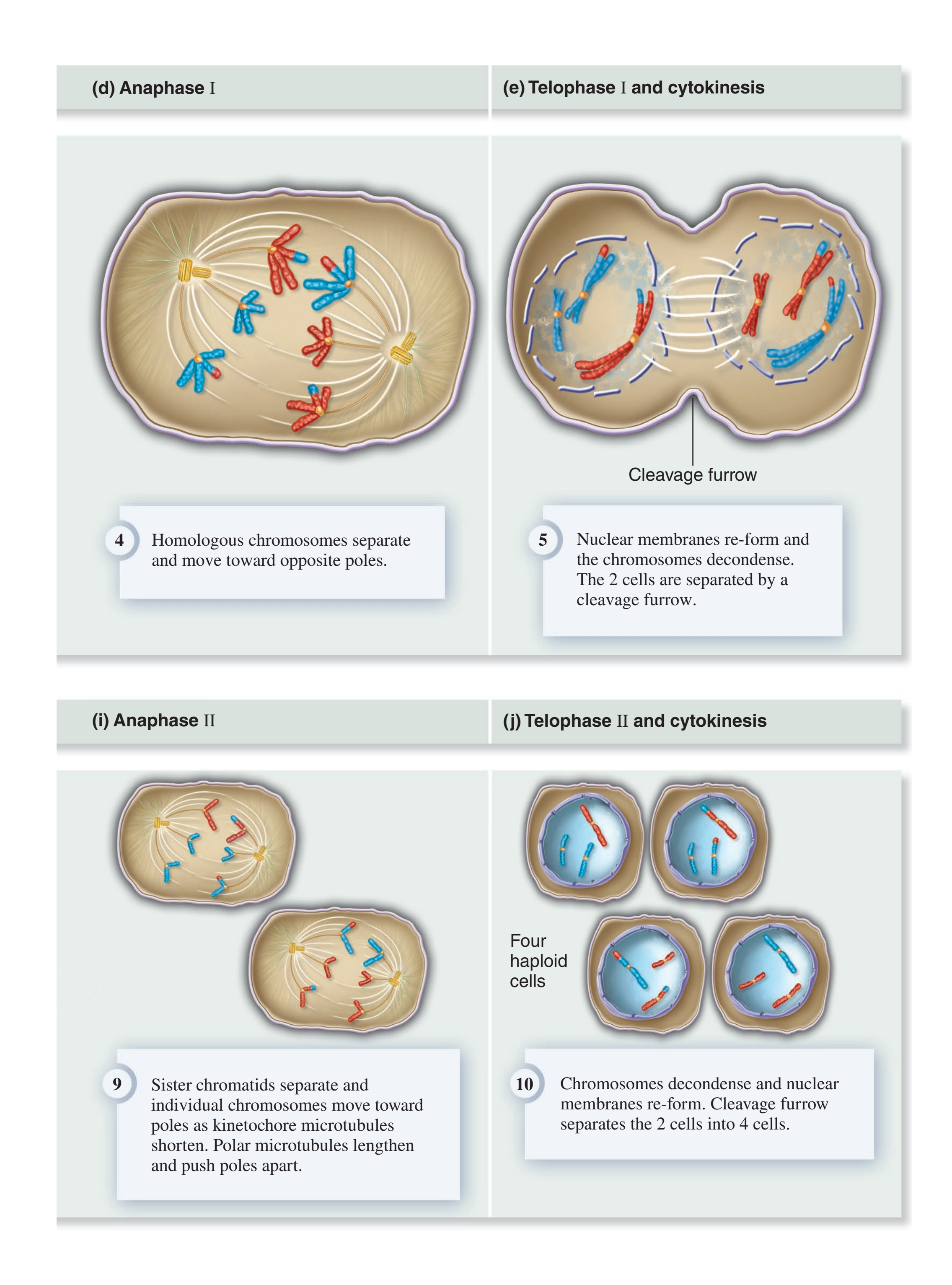
Figure 15.4 Stages of meiosis.

11. Chromosomal events are a continuous process rather than distinct steps. Therefore, repeat steps 4–10 as a continuous process and ask your instructor to verify your simulation.

GAMETOGENESIS

Meiosis occurs in all sexually reproducing eukaryotes and produces haploid nuclei. However, organisms vary in the timing and structures associated with producing functional gametes. **Gametes** are reproductive cells with haploid nuclei resulting from meiosis, and the formation of gametes is called **gametogenesis.** Meiosis is the primary element of gametogenesis in animals, but after meiosis the cells must mature and usually change their morphology before becoming a functional gamete.

In this exercise, you will examine mammalian gametogenesis. Gametogenesis includes **spermatogenesis,** the



formation of sperm cells, and **oogenesis,** the formation of egg cells (fig. 15.5).

Mammalian Spermatogenesis

Spermatogenesis occurs in male testes made of tightly coiled tubes called **seminiferous tubules** (fig. 15.6). Examine a prepared slide of a cross-section through the seminiferous tubules of a monkey, rat, or grasshopper. Packed against the inner walls of the tubules are diploid cells called **spermatogonia.** These cells constantly replicate mitotically during the life of males. They are assisted by nongerminal cells called **sertoli cells.** Some of the daughter cells move inward toward the lumen of the tubule and begin meiosis. These cells are called **primary spermatocytes.** Meiosis I of a primary spermatocyte produces two **secondary spermatocytes,** each with a haploid set of double-stranded chromosomes.

Meiosis II separates the strands of each chromosome and produces two haploid cells called **spermatids.** Spermatids mature and differentiate into **sperm** cells as they move

Table 15.1

Events of Meiosis

Events of Meiosis
Prophase I
Prometaphase/Metaphase I
Anaphase I
Telophase I
Prophase II
Prometaphase/Metaphase II
Anaphase II
Telophase II

Sperm cells by the numbers

- Healthy males produce 100–200 million sperm cells per day.
- There are an estimated 250 million sperm cells per ejaculation.
- There are 200–300 million sperm cells for every one egg.
- There are about 20 million sperm/mL of semen.
- About 20% of all sperm are deformed.
- Sperm can live inside a female reproductive system for up to 6 or 7 days.
- Conception can occur several days after sexual intercourse.

along the length of the tubule. Review these basic stages of spermatogenesis in figure 15.5. Then examine some prepared slides of sperm cells from vertebrates such as guinea pig, rat, and human.

Question 4

a. During gametogenesis a sperm cell undergoes considerable structural change. What are the basics of sperm structure and how do these features relate to function?

b. What is the advantage of producing sperm in a system of tubes rather than in solid tissue?

c. What is each strand of a double-stranded chromosome called?

Spermatogonium (2*n*)

DNA replication

Primary spermatocyte (2*n*)

Meiosis I

Secondary spermatocyte (*n*)

Meiosis II

Spermatids (*n*)

Mature sperm cells (*n*)

(a) Spermatogenesis (males)

Oogonium (2*n*)

DNA replication

Primary oocyte (2*n*)

Meiosis I

Secondary oocyte (*n*)

First polar body (*n*)

Degenerates

Meiosis II

Second polar body (*n*)

Mature ovum (*n*)

Degenerates

(b) Oogenesis (females)

Figure 15.5 Gametogenesis in (*a*) males and (*b*) females. Both male and female germ cells are diploid (2*n*) cells that undergo two meiotic divisions to produce mature haploid (*n*) gametes.

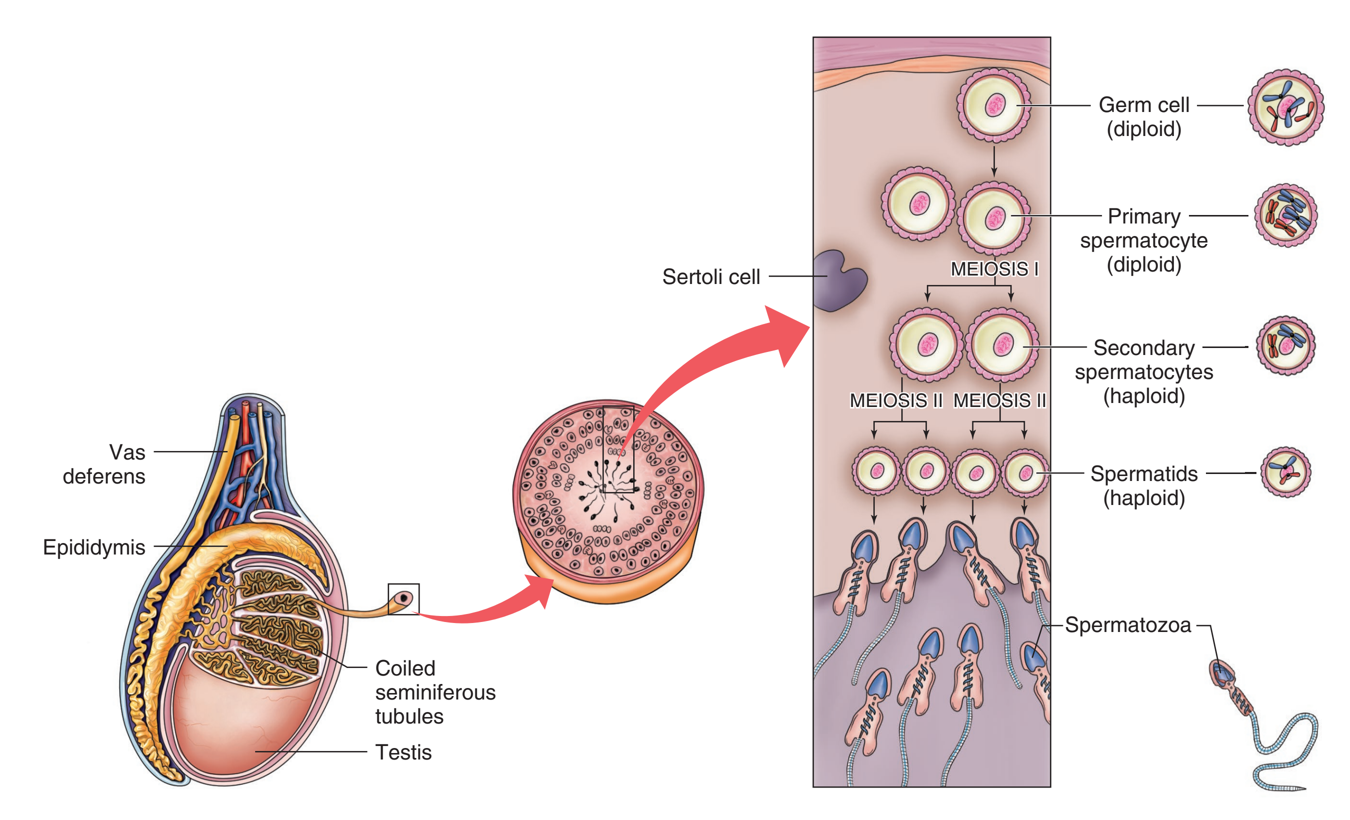


Figure 15.6 The interior of the testis, the site of spermatogenesis. Within the seminiferous tubules of the testis, germinal cells called spermatogonia pass through the spermatocyte and spermatid stages to develop into sperm. Each sperm possesses a long tail coupled to a head, which contains a haploid nucleus.

Mammalian Oogenesis

Oogenesis occurs in ovaries of females (fig. 15.7). Cells of the ovary that produce female gametes are called **oocytes.** However, oocytes are not produced continually by the ovary, as spermatocytes are produced by the testes. During early fetal development, **oogonia** (germinal cell) are produced in the ovaries. These oogonia replicate mitotically to produce as many as two million **primary follicles,** each containing a **primary oocyte.** In humans, ovaries of a newborn female contain all of the primary oocytes that she will ever have (i.e., oogonia produce no more primary oocytes). At birth the primary oocytes in a female have begun meiosis I but are arrested in prophase I. They are surrounded by supportive **follicular cells,** and together they are called **follicles.** At puberty, circulating hormones stimulate growth of one or two of these dormant follicles (and their primary oocytes) each month. The oocyte enlarges and the number of follicular cells increases. Just before **ovulation** (release of the oocyte from the ovary) the oocyte completes meiosis I, which produces a **secondary oocyte** and a **polar body.** This mature follicle is called a **Graafian follicle** and contains a secondary oocyte (fig. 15.8). Each secondary oocyte contains a haploid set of double-stranded chromosomes (two chromatids), but cytoplasmic cleavage is unequal. The secondary oocyte retains most of the cytoplasm and the first polar body usually disintegrates.

Examine a prepared slide of a mammalian ovary cross-section. In the following space sketch a Graafian follicle and two or three less mature stages.

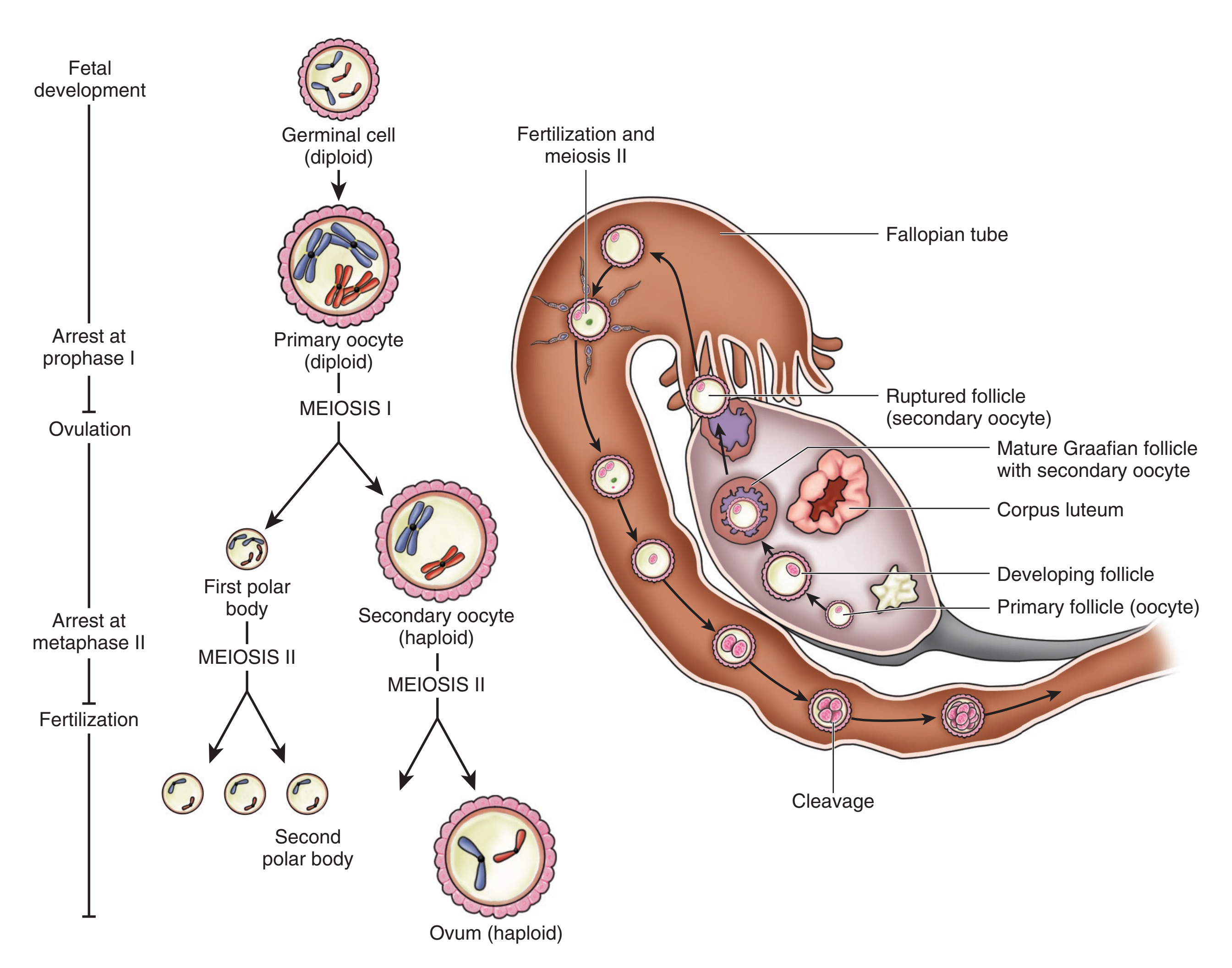
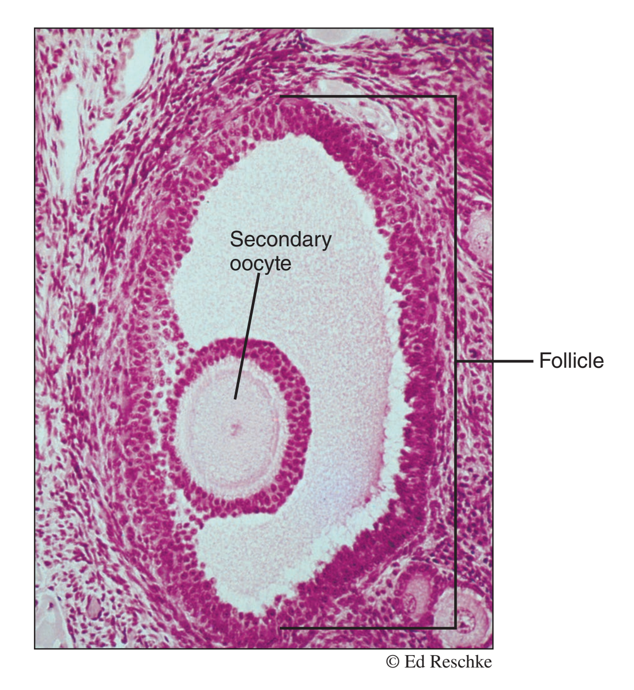


Figure 15.7 Oogenesis. A primary oocyte is diploid ($2n$). After its first meiotic division, one product is eliminated as a polar body. The other product, the secondary oocyte, is released during ovulation. Sperm penetration stimulates the second meiotic division, and a second polar body and a haploid ovum are produced. Fusion of the haploid (n) ovum nucleus with a haploid (n) sperm nucleus produces a diploid ($2n$) zygote that subsequently forms an embryo.



© Ed Reschke

Figure 15.8 A mature secondary oocyte in an ovarian follicle of a cat (400×). This secondary oocyte awaits ovulation.

Question 5
How would retaining extra cytoplasm enhance survival of a developing oocyte?

Meiosis II proceeds but is not completed until after a sperm cell penetrates the egg. Completion of meiosis II produces another polar body and a haploid egg cell ready for **fertilization** (fusion of nuclei). Review these basic stages of oogenesis in figure 15.5. Then examine a cross-section of a cat ovary.

Question 6
a. What are the relative sizes of oocytes in a dormant follicle, a growing follicle, and a Graafian follicle?

b. Are polar bodies visible in your prepared slide of a cat ovary? Why or why not?

After ovulation the remaining follicle cells form the **corpus luteum** on the surface of the ovary. The corpus luteum produces hormones that prepare the uterus for the potential arrival of a fertilized egg.

Plant Gametogenesis

The formation of gametes in plants is somewhat different because their sexual life cycle includes an alternation of generations between haploid and diploid forms. However, meiosis is still the critical process by which plants reduce the number of chromosomes by half to prepare for gamete production.

In flowering plants, meiosis occurs in the anthers and ovary of the flowers. In the anther, the spores resulting from meiosis produce a stage of the life cycle (pollen) that will eventually produce male gametes. In the ovary, the spores resulting from meiosis produce a stage of the life cycle (ovule) that will eventually produce female gametes. You'll learn more about these events in Exercise 31. In this procedure, you will observe prepared slides showing stages of the beginning, middle, and end of meiosis I and II in a representative plant.

Procedure 15.2 Diagram and observe stages of meiosis

1. In figure 15.9, diagram a plant cell with three pairs of chromosomes in each of the stages of meiosis. Be sure to label the cell wall and cell plate.
2. Examine the following prepared slides of stages of meiosis in a *Lilium* anther (see figs. 31.10, 31.11).
   a. *Lilium* anther—early prophase I
   b. *Lilium* anther—late prophase I
   c. *Lilium* anther—first meiotic division
   d. *Lilium* anther—second meiotic division
   e. *Lilium* anther—pollen tetrads. Each of these cells will produce a pollen grain.
3. Examine the following prepared slides of stages of meiosis in a *Lilium* ovary.
   a. *Lilium* ovary—"mother cell," prophase I
   b. *Lilium* ovary—binucleate stage, end of meiosis I
   c. *Lilium* ovary—four nucleate stage, end of meiosis II

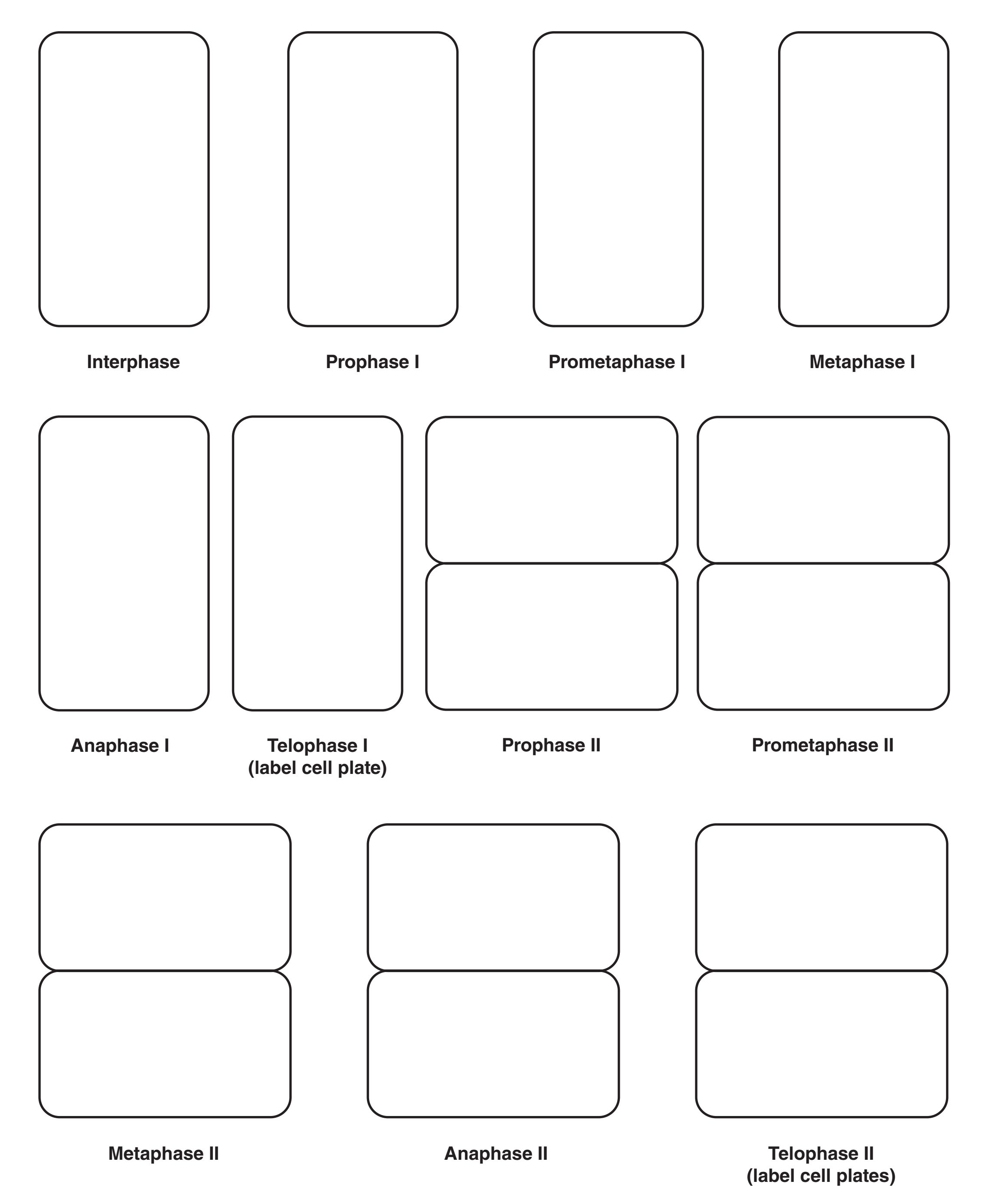


Figure 15.9 Stages of meiosis in plants.

MITOSIS VERSUS MEIOSIS

Mitosis and meiosis are both forms of cellular replication but they play different roles in the life cycle of animals and plants. Mitosis may occur in either haploid or diploid cells and is necessary for cell production and growth. Meiosis occurs in diploid cells. Its role is to produce cells with a reduced number of chromosomes and shuffle the genetic material so an organism can reproduce sexually. To compare mitosis and meiosis, review table 15.2 and complete the column with the contrasting features of meiosis.

INVESTIGATION

Variation in the Morphology of Vertebrate Sperm Cells

Observation: The morphology of sperm cells directly relates to their function. Sperm of vertebrates such as guinea pigs, rats, and humans vary in size and shape.

Question: How does sperm cell morphology vary among species of vertebrates?

a. Establish a working lab group and obtain Investigation Worksheet 15 from your instructor.

b. Discuss with your group the measurements and observations you might make to reveal variation in sperm morphology. Pose a well-defined question relevant to the preceding observation and question. Record it on Worksheet 15.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 15 the procedures and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Table 15.2

A Comparison of the Major Features of Mitosis and Meiosis

	Mitosis	Meiosis
Purpose of process		
Number of cells produced		
Number of nuclear divisions		
Haploidy or diploidy of resulting cells		
Genetically identical cells (yes or no)		
Pairing of homologues (yes or no)		
Occurrence of crossing over (yes or no)		

Questions for Further Thought and Study

1. How would you diagram the chromosomal arrangement for transitional stages such as late prophase/early metaphase I? Or late anaphase/early telophase I?

2. Would evolution occur without the events of meiosis and sexual reproduction? Why or why not?

3. What are the general characteristics of sexual reproduction in humans and other vertebrates that are associated with continuous production of many sperm cells but intermittent, finite production of egg cells?

4. Which process is most accurately referred to as nuclear division: meiosis or mitosis?

5. What special event does interkinesis lack compared to premeiotic interphase?

6. How are mammalian sperm cells produced and incubated at a lower temperature than body temperature?

7. How old is an ovulated oocyte of a 35-year-old woman? What consequences does this have?

WRITING TO LEARN BIOLOGY

Wouldn't it be easier for a cell simply to divide the chromosomes once rather than duplicating them and then dividing them twice during meiosis? Why do you suppose this isn't done?

Molecular Biology and Biotechnology

DNA Isolation and Genetic Transformation

Learning Objectives

By the end of this exercise you should be able to:

1. Isolate DNA from a bacterium.
2. Understand how temperature and pH affect DNA.
3. Insert a gene for resistance to ampicillin into a bacterium.



Please visit connect.mheducation.com to review online resources tailored to this lab.

Biotechnology is the manipulation of organisms to do practical things and to provide useful products. Biotechnology has been around for centuries: humans have selectively bred livestock for meat products, controlled pollination to produce more food, and used bacteria and fungi to make wine and cheese. But recent progress in molecular biology has revolutionized biotechnology, and its products include vaccines, detergents, drugs, biodegradable plastics, alcoholic beverages, industrial chemicals (e.g., ethanol, acetone), antibiotics, hybrid crops, livestock food, cooking oils, improved textiles and fabrics, lower-cholesterol meats and eggs, and many common foods (e.g., corn, watermelon, chicken, cheese) and beverages (e.g., milk, beer, wine). The revolution stems from new molecular techniques that have made genetic engineering possible.

Genetic engineering is the direct manipulation of genes for practical purposes (fig. 16.1). Genetic engineers can intervene *directly* in the genetic fate of organisms. We can isolate genes, move them from one organism to the next, and even move genes from one species to the next. The most common goals of this engineering are to harvest the valuable proteins made by engineered genes and to benefit from the new characteristics the genes provide to the target organisms, including humans. Indeed, the impact on society of the current revolution in genetic engineering may soon surpass that of such historical changes as the industrial revolution of the past two centuries.

Genetic engineering got its start in 1973 when Stan Cohen and Herb Boyer transplanted a gene for antibiotic resistance from a frog into a bacterium, and thus "engineered" an antibiotic-resistant organism. In 1980, molecular biologists successfully inserted a human gene for interferon, an antiviral drug, into a bacterium. When the "transformed" bacterium reproduced, it generated billions of progeny, each a miniature drug factory. As a result of this engineering, biologists could cheaply harvest a drug that was previously expensive and generally unavailable. Genetic engineering has since been applied to medicine (gene therapy, drug production) and the production of new foods and environmentally benign pesticides. Today, millions of diabetics worldwide use synthetic human-insulin to regulate their blood-sugar levels. Insulin is made by genetically engineered bacteria and yeast.



Figure 16.1 The United States is the world's top per-capita consumer of genetically modified crops, and the production of these crops demonstrates the importance of genetic engineering in our lives. For example, in 1996, only 8% of the cropland devoted to corn was used to grow genetically engineered corn; by 2014, this percentage had increased to 93%. Yields also increased, from 127 bushels per acre in 1996 to 160 bushels per barrel in 2014. The 14 billion bushels of corn harvested in 2014 were eaten and used to make ethanol, beverages, dyes, adhesives, tires, drugs, and countless other products.

At the heart of genetic engineering is the science of **molecular biology** (i.e., the study of molecules critical to life). Molecular biologists recently have concentrated their efforts on manipulating "information molecules" such as DNA and proteins because all outward characteristics of

organisms have their basis in proteins from genes made of DNA. In addition to providing techniques for genetic engineering, molecular biology has also impacted fields such as forensics (e.g., linking suspects to crimes, settling paternity disputes), hiring practices (pinpointing employees at high risk for cancer), and agriculture (e.g., in 2014, more than 17 million farmers in 27 countries planted biotech crops on more than 430 million acres of farmland). In the United States, genetically modified crops constituted 95% of the nation's sugar beets, 93% of the feed corn, 96% of the cotton, and 94% of the soybeans in 2014. To introduce yourself to the core information of molecular biology, you should review DNA structure in your textbook and in Exercise 6, "Biologically Important Molecules."

In this exercise you'll learn two techniques used routinely by molecular biologists: (1) isolation of DNA, and (2) genetic transformation.

ISOLATION OF DNA

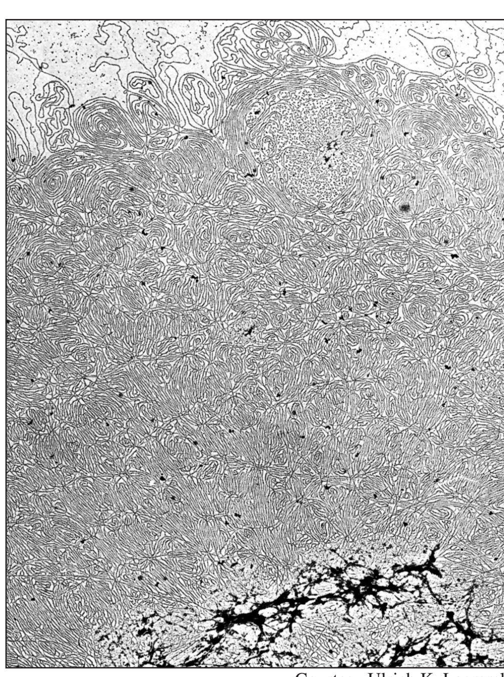
Isolation of DNA is a routine and important procedure for molecular biologists. Once isolated, the DNA and the sequence of its subunits can be determined, manipulated, or altered. Bacteria each contain only about 10^{-14} g of DNA, which accounts for approximately 5% of the organism's dry weight. However, molecules of this DNA can be very long; for example, the DNA in an *E. coli,* if strung out, would be approximately 1 mm long. (By analogy, if the bacterium were the size of a grapefruit, then its DNA would be more than 80 km long.) The ability to pack this much DNA into a tiny cell is impressive, especially because DNA is a rather stiff molecule (fig. 16.2).

When DNA or other large molecules are isolated from cells, the surrounding solution becomes viscous (i.e., thick, syrupy, and resistant to flow). This is because DNA molecules are long and tend to stick to each other due to cohesion among molecules and hydrogen bonding (recall that hydrogen bonding also holds the two strands of DNA's double helix together; see fig. 6.9). Harsh chemicals will nonspecifically disrupt hydrogen bonds, including those between corresponding nitrogenous bases of DNA. Molecular collisions produced by high heat can tear molecules apart, and any breakdown in molecular structure will reduce viscosity.

In this exercise, you will isolate DNA from *Halobacterium salinarum,* a halophilic ("salt-loving") bacterium that grows only in salty environments (4–5 M NaCl). It's especially easy to isolate DNA from this organism because its cell walls disintegrate when placed in low-salt environments (0–2 M NaCl).



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.



Courtesy Ulrich K. Laemmli

Figure 16.2 A human chromosome contains an enormous amount of DNA (35,000×). The dark element at the bottom of the photograph is part of the protein matrix of a single chromosome. All of the surrounding material is the DNA of the chromosome.

Procedure 16.1 Isolate DNA from *Halobacterium salinarum*

1. Several days ago your laboratory instructor inoculated petri dishes of culture media with *Halobacterium salinarum.* Obtain one of these cultures from your instructor.
2. Use a flexible plastic ruler to scrape the bacterial growth from the surface of the agar and collect it in a test tube.
3. Add 1 mL of distilled water to the tube. One milliliter equals about 20 drops.
4. Place a small piece of plastic film over the top of the test tube and hold it securely in place with your thumb. Invert the tube several times to mix the contents. This mixing with water lyses the cells, and the liquid will become viscous.
5. Use an eyedropper to slowly and gently pour 1 mL of ice-cold 95% ethanol down the side of the tube. Do not shake or disturb the tube. DNA will precipitate at the interface between the layers of water and ethanol.
6. Insert a glass rod into the liquid. Rotate the rod. DNA will adhere to the rod as it is twirled.

Question 1

What is the texture of the DNA you've isolated?

The DNA that you've isolated is not pure; rather, it is contaminated with small amounts of protein and RNA.

ISOLATE DNA FROM YOUR CELLS

If time permits, your instructor may also ask you to extract DNA from your cheek cells. These procedures will work best if you have not eaten or chewed gum lately.

Examine the cells from which you will extract your DNA.

1. Place a drop of methylene blue on a clean microscope slide. Be careful; methylene blue will stain your skin and clothes.
2. Gently scrape the inside of your cheek with the flat side of a toothpick. Swirl this end of the toothpick in the drop of methylene blue. Then throw the toothpick away.
3. Place a coverslip on the drop of methylene blue and examine the slide with your light microscope.

At low power, cells will appear as small, purplish objects. After finding some of these cells, examine the cells with a higher-power objective. These are the cells from which you will extract your DNA.

Extracting your DNA

You'll extract your DNA by (1) collecting your cells, (2) releasing the DNA by using detergent to lyse the cell and nuclear membranes of your cells, and (3) precipitating your DNA by adding alcohol.

1. Pour 10 mL of a lightly colored sports-drink (or a 0.9% solution of sodium chloride) into a small, disposable cup.
2. Collect your cheek cells by *vigorously* swirling the sports drink or salt solution in your mouth for 1 min. While swirling the solution in your mouth, rub your cheek with your tongue and teeth. Cells lining your mouth are easily loosened, and this swirling will dislodge cells, which will be the source of your DNA. The longer and more vigorously you swirl the solution in your mouth, the more cells and DNA you will collect. (This solution will also contain bacteria from your mouth, from which you will also isolate the DNA.)
3. Spit the sports-drink solution back into the disposable cup.
4. Pour the sports-drink solution from the cup into a test tube containing 5 mL of a 25% solution of dishwashing detergent. The detergent will break the lipid-based cell and nuclear membranes, thereby releasing the cells' DNA into solution. (This is why we use detergents to remove fats—that is, lipids—from dirty dishes.)
5. Add a pinch of meat tenderizer to the test tube. Each of our cells contains enough DNA which, if stretched end-to-end, would span 2–3 m. To fit this much DNA into a tiny cell, the DNA is wrapped tightly around proteins. Meat tenderizer is a protease, which is an enzyme that digests protein and, in doing so, releases DNA from the protein.
6. Cap or cork the test tube and *gently* rock the tube from side to side for 3 min. Also *gently* invert the tube 4–5 times. Do not vigorously shake or tilt the tube; doing so will shear the DNA into smaller pieces that will be hard to see later. If caps or corks are not available, you can also cover the end of the test tube with your gloved thumb or a piece of Parafilm.
7. Let the tube stand for 1 min.
8. After uncapping the tube, gently tilt the tube to a 45° angle and use a pipette to gently add 10 mL of ice-cold ethanol down the side of the tube. (The colder the alcohol, the more DNA will precipitate.) Alcohol is less dense than the detergent solution, so the alcohol will form a layer atop the detergent.
9. Let the tube stand for 10 min. Do not shake, tip, mix, or agitate the tube. Watch what happens at the interface between the alcohol and detergent.
10. Use a glass rod to slowly move some of the alcohol into the detergent. When you do this, your DNA—which is insoluble in alcohol—will precipitate as white, cottony strands at the alcohol-detergent interface. The lipids and proteins will remain dissolved. Bubbles displaced from the sports drink may get trapped in the DNA and make it easier to see.
11. Spool your DNA on the glass rod by slowly twirling the rod in one direction.

The Influence of Heat and pH on DNA

Heat and pH strongly affect the properties of DNA. For example, DNA typically denatures at alkaline pH and at 80–97°C. Test these effects with the following procedures.

Procedure 16.2 **Test the influence of heat on DNA**

1. Precipitate DNA in a test tube following steps 1–5 in procedure 16.1 for isolating DNA.
2. Place the tube into a boiling water-bath for 10 min.
3. Place the tube in an ice bath.
4. Insert and twirl a glass rod in the tube.
5. Compare the viscosity of the heat-treated DNA with untreated DNA.

Question 2

a. What effect does heat have on the viscosity of DNA?

b. What do you think is the mechanism for this change in viscosity?

Procedure 16.3 **Test the influence of pH on DNA**

1. Precipitate DNA in a test tube following steps 1–5 in procedure 16.1 for isolating DNA.
2. Add 2 mL of 1.0 N NaOH to the tube.



NaOH is caustic. Don't spill it on yourself or your clothes.

3. Insert and twirl a glass rod in the tube.
4. Compare the viscosity of the alkali-treated DNA with untreated DNA and with heat-treated DNA.

Question 3

a. What effect does alkaline pH have on the viscosity of DNA?

b. What do you think is the mechanism for this pH-induced change in viscosity?

GENETIC TRANSFORMATION

Much of biotechnology is based on **genetic transformation,** which is the uptake and expression of DNA by a living cell. A successful transformation, summarized in figure 16.3, requires three conditions: (1) a host into which DNA can be inserted, (2) a means of carrying the DNA into the host, and (3) a method for selecting and isolating the successfully transformed organisms.

The Host: *Escherichia coli*

The **host organism** you'll use is the bacterium *Escherichia coli,* one of the most intensively studied organisms in the world. *E. coli* has the following properties that make it ideally suited for transformation:

- It contains only one chromosome made of five million base-pairs. This is less than 0.2% of that of the human genome.
- *E. coli* grows rapidly. Transformations in bacteria are rare and occur in only about 0.1% of cells. Therefore, transformations are observed most easily in large, rapidly growing populations. *E. coli* is ideal for transformation studies because, in ideal conditions, it divides every 20 min. As a result, in 10 h a bacterium can produce a billion progeny (30 generations) in only 1 mL of nutrient broth.

Only a small percentage of bacterial cells in a culture can be transformed. Also, small lengths of DNA are taken up more readily than long lengths. However, competence of the bacteria (i.e., the chances for successful transformation) increases during the early and middle stages of its growth. Competence also increases when cells suspended in a cold solution of $CaCl_2$ are heat-shocked. Yield is usually about 10^6 transformants per milligram of DNA available for insertion.

A Vector to Move DNA into the Host

A biological **vector** is a DNA molecule that carries DNA sequences into a host. The simplest bacterial vectors are **plasmids,** circular pieces of DNA made of 1000 to 200,000 base-pairs (fig. 16.4). Plasmids exist separately from the

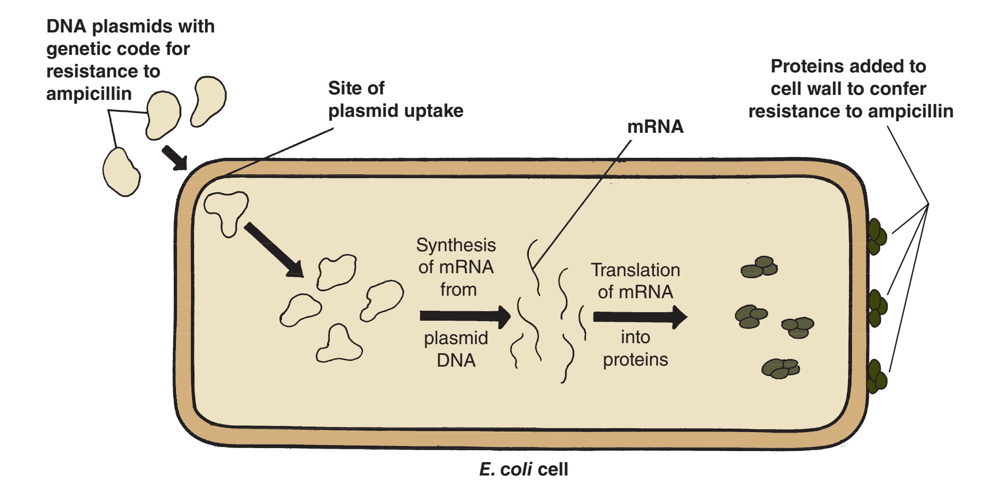
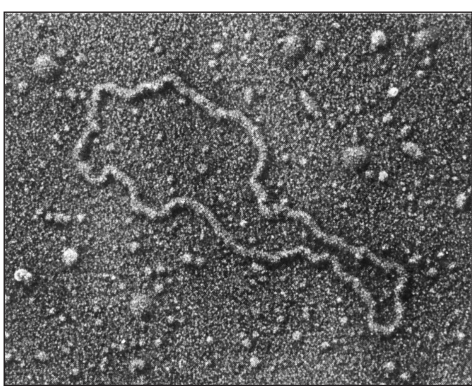


Figure 16.3 Transformation of an *E. coli* cell with plasmid DNA. In this example, the DNA plasmid contains the genetic code for resistance to the antibiotic ampicillin. After uptake of the plasmid, the code is transcribed to messenger RNA, which is translated during protein synthesis. Addition of these proteins to the cell wall will retard attack by ampicillin.



Courtesy Dr. Stanley N. Cohen

Figure 16.4 A famous plasmid. The circular molecule in this electron micrograph (70,000×) was the first plasmid used successfully to clone a vertebrate gene, pSC101. Its name refers to it being the 101st plasmid isolated by Stanley Cohen.

bacterial chromosome, and they must contain a gene that confers some selective advantage (e.g., resistance to an antibiotic) to remain in the host. We don't completely understand how plasmids enter host cells, but they seem to enter consistently.

Selecting Transformed Organisms

You'll insert into *E. coli* a plasmid (pAMP) containing a gene for resistance to ampicillin, an antibiotic lethal to many bacteria (fig. 16.5). (Refer to Exercise 24 for information on bacterial cell wall structure and how ampicillin might kill bacteria.) Then you'll select transformed bacteria based on their resistance to ampicillin by spreading the transformed organisms onto nutrient medium containing ampicillin. Organisms that grow on this medium have been transformed. Because *E. coli* grows so fast, you can check for transformed organisms only 12–24 h after completing the experiment.

Procedure 16.4 Transform *E. coli*

Preparation

1. Carefully read this procedure and review figure 16.5 before beginning. Wash your hands. Your instructor will demonstrate and discuss **sterile technique,** which will eliminate contamination of your cultures by other organisms (e.g., bacteria, fungi). Use sterile technique when doing this procedure.
2. Fill a 250-mL beaker with about 50 mL of ethanol and place a glass-rod bacterial spreader in the ethanol to soak.

Preincubation to Increase Competency

3. Obtain a test tube with 1 mL of sterile, yellow nutrient broth, and a tube with 1 mL of clear, colorless 50 mM $CaCl_2$. Label these tubes NB and $CaCl_2$, respectively.
4. Fill a 250-mL beaker half full with crushed ice. Place the tube of $CaCl_2$ in the beaker of ice.
5. Obtain two sterile, plastic transformation tubes and label one of them (+)P, meaning with plasmid, and the other (−)P, meaning without plasmid. Place them in the beaker of ice.
6. Obtain a packaged, sterile, plastic pipet, and locate the graduation indicating a volume of 0.25 mL.
7. Open the packaged pipet without touching and contaminating the pipet's open end. Use this pipet to add 0.25 mL of a sterile, ice-cold solution of 50 mM $CaCl_2$ to each of the two transformation tubes. Use sterile technique. This is shown in step 1 of figure 16.5.
8. Several days ago your lab instructor streaked starter plates of nutrient agar with *E. coli.* Scrape a colony of *E. coli* (3-mm diameter) from one of these plates with a sterile, plastic inoculating loop. The bacteria are growing as a thin film on the surface of the agar; when you scrape a colony off the surface, be sure not to take up any of the agar. Use sterile technique. This is shown in step 2 of figure 16.5.
9. Place the loopful of bacteria into the transformation tube labeled (−)P. Rinse the bacteria from the loop by gently twirling the loop handle between your fingers.
10. To mix the bacterial suspension, open a sterile, packaged pipet. Insert the pipet into the suspension in the bottom of the tube and gently use a rubber bulb to suck the fluid in and out of the tip three or four times. Be sure the tip is empty before withdrawing the pipet.
11. For tube (+)P, repeat steps 8–10. Use a fresh loop and pipet to inoculate and mix the bacteria. Try to get the same amount of bacteria into each tube. Replace the two tubes in the ice bath and chill the tubes for at least 5 min.

Incubation

12. Use a sterile loop to obtain one loopful (10 µL) of an ice-cold solution of DNA plasmids from a vial kept by your instructor. Add this loopful of plasmids to tube (+)P and gently rotate the loop to rinse the plasmids from the loop. These plasmids contain the gene for resistance to ampicillin. Do **not** add plasmids to tube (−)P. This is shown in step 3 of figure 16.5.
13. Place both tubes in ice for 15 min.
14. While the tubes are cooling, obtain two agar plates labeled (−)AM, meaning nutrient agar without ampicillin, and two plates labeled (+)AM, meaning nutrient agar with ampicillin. Label one of each pair of plates as (−)P and the other two plates as (+)P.

Heat Shock

15. Heat-shock the transformation tubes (−)P and (+)P by placing them in a 42°C water-bath for 2 min. Shake the tubes while they are in the water-bath. Heat shock increases the uptake of the plasmid by the bacterial cells.
16. Chill the tubes in ice for 5 min.
17. Remove the tubes from the ice bath; place them in a test-tube rack or empty beaker.

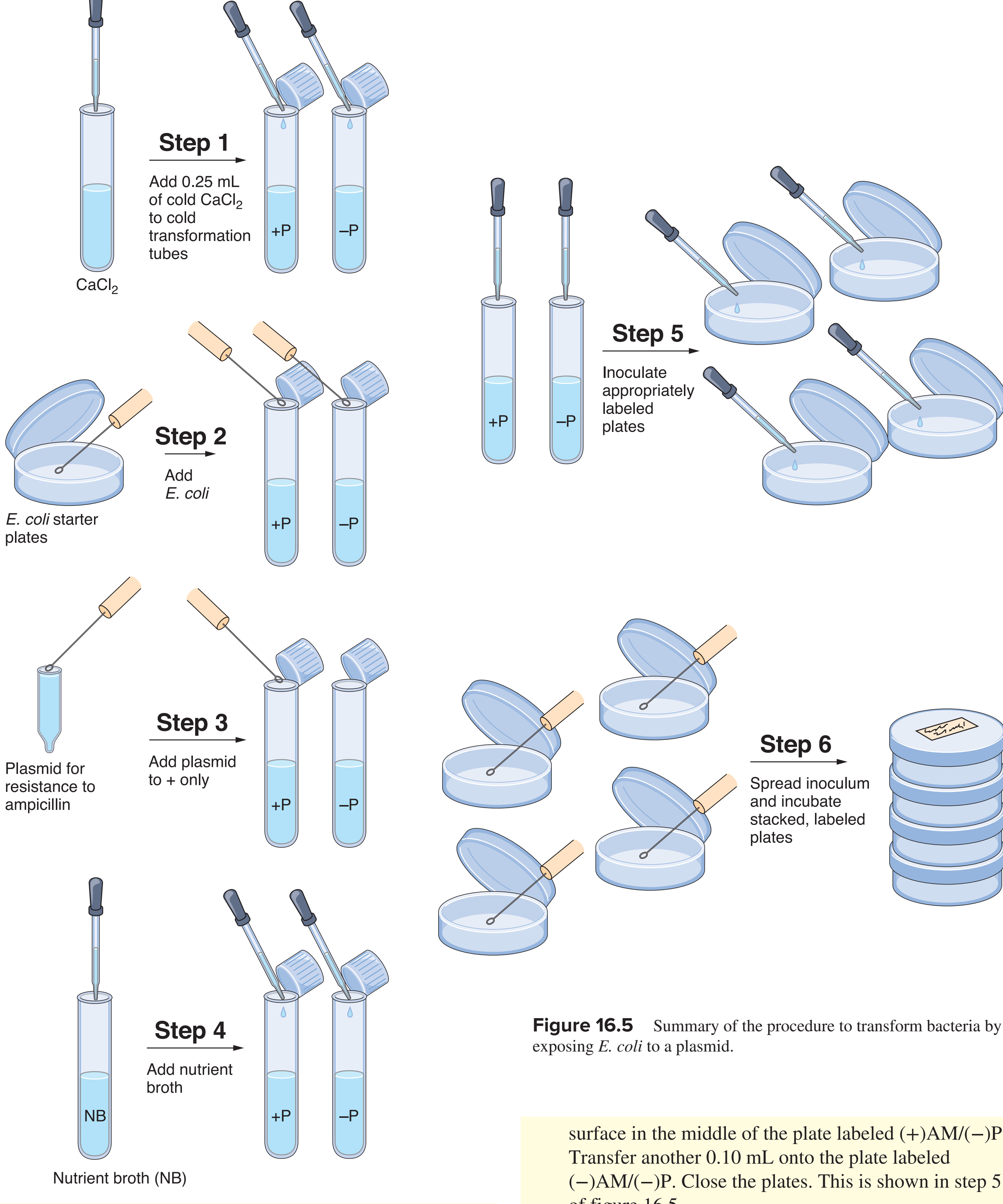


Figure 16.5 Summary of the procedure to transform bacteria by exposing *E. coli* to a plasmid.

Recovery and Plating the Samples

18. Using a sterile pipet, add 0.25 mL nutrient broth to each tube and tap gently to mix the contents. This is shown in step 4 of figure 16.5.

19. Using sterile technique and a sterile pipet, transfer 0.10 mL of the (−)P cell suspension onto the agar's surface in the middle of the plate labeled (+)AM/(−)P. Transfer another 0.10 mL onto the plate labeled (−)AM/(−)P. Close the plates. This is shown in step 5 of figure 16.5.

20. Using another sterile pipet, transfer 0.10 mL of the (+)P cell suspension onto the middle of the plate labeled (+)AM/(+)P. Transfer another 0.10 mL onto the plate labeled (−)AM/(+)P. Close the plates.

21. Light an alcohol lamp. Dip the bacteria spreader into the ethanol and then into the flame of an alcohol lamp.

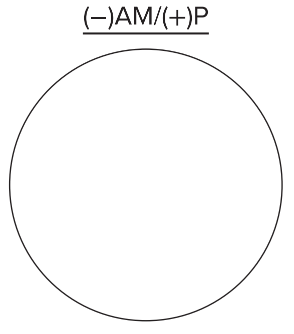
Let the ethanol burn away; then count to 10 to let the spreader cool. Before spreading the bacteria in the first of your four plates, further cool the spreader by touching it to the agar at the edge of the plate. Then touch the spreader to the cell suspension in the middle of the plate and gently drag it back and forth three times. Rotate the plate 90° and repeat. This is shown in step 6 of figure 16.5. Remember to sterilize the bacteria spreader between each plate. Repeat this procedure to spread the bacteria on the other three plates.

Selecting the Transformed Organisms

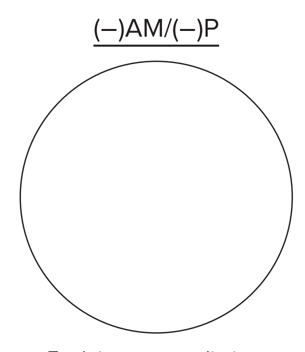
22. Put your name and date on each of the four plates, and tape the plates together. Incubate the plates upside down at 37°C.

23. Place all the tubes, loops, and other such materials, in a central location for disposal. Wipe your work area with a weak bleach solution and wash your hands before leaving the laboratory.

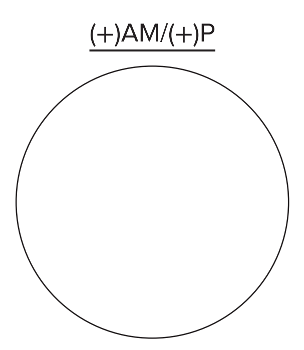
24. In the space below indicate your predictions for growth (+) or no growth (−). Explain your reasoning for each prediction in the provided space.



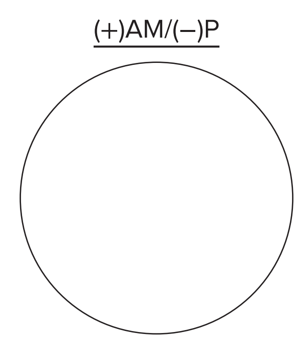
Explain your prediction:



Explain your prediction:

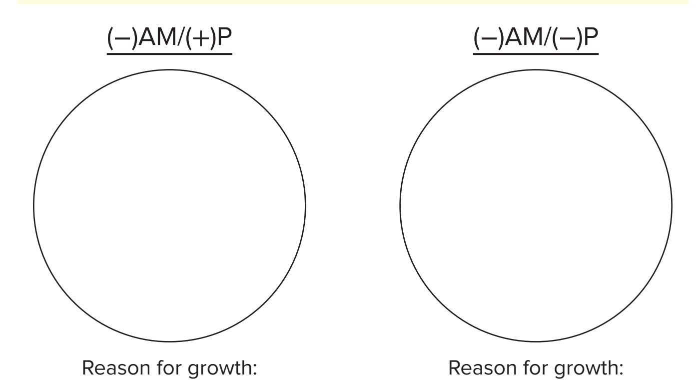


Explain your prediction:



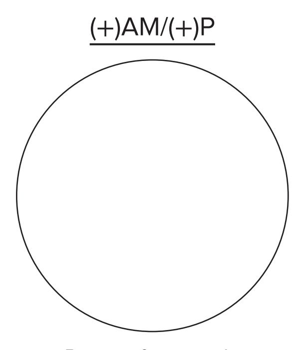
Explain your prediction:

25. In the space below indicate with a (+) which plates had bacterial growth after 24 h. Draw the appearance and coverage of bacterial colonies and explain possible reasons for growth *and* possible reasons for no growth.



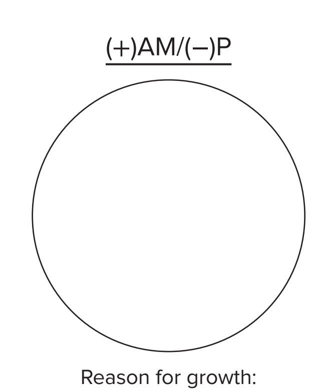
Reason for growth: | Reason for growth:

Reason for no growth: | Reason for no growth:



Reason for growth:

Reason for no growth:



Reason for growth:

Reason for no growth:

Question 4

a. Which treatment produced transformed bacteria?

b. How many transformed colonies grew on each plate?

c. What was the purpose of tube 2 without plasmid?

INVESTIGATION

Antibiotic Resistance by Transformed Bacteria

Observation: In this lab you inserted a plasmid into the bacterium *Escherichia coli*. That plasmid conferred resistance to the antibiotic ampicillin.

Question: Are transformed bacteria that are resistant to ampicillin also resistant to other antibiotics?

a. Establish a working lab group and obtain Investigation Worksheet 16 from your instructor.

b. Discuss with your group well-defined questions relevant to the preceding observation and question. Choose and record your group's best question for investigation.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 16 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypothesis, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. Consider the ubiquitous occurrence of bacteria in nature, along with the constant fragmentation and release of DNA as cells decompose. How frequently do genetic transformations occur in nature? Explain your answer.

2. How could genetic transformations improve our quality of life? How could they decrease our quality of life?

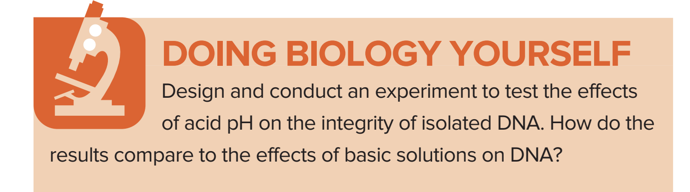
3. Why is molecular biology often referred to as genetic engineering or biotechnology?

4. How could the uptake of plasmids in natural systems be important?

5. The development of antibiotic resistance is a major threat to our health. Why? How extensive is this problem?

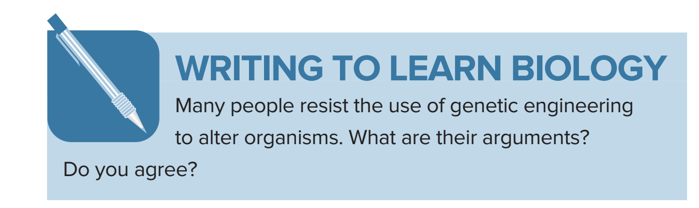
6. In 1997, only 10% of the U.S. cotton crop was genetically engineered, but that percentage rose to 94% in 2014. Similarly, only 17% of the U.S. soybean crop was genetically engineered in 1997, but that percentage rose to 94% in 2014. Why are genetically engineered crops becoming so popular in the United States?

7. As of 2014, only one crop—a type of corn engineered to fight off pests such as the European corn borer—was approved for cultivation in the European Union. What concerns have limited the impact of genetically engineered crops in Europe? Do you consider these concerns to be valid? Why or why not?



DOING BIOLOGY YOURSELF

Design and conduct an experiment to test the effects of acid pH on the integrity of isolated DNA. How do the results compare to the effects of basic solutions on DNA?



WRITING TO LEARN BIOLOGY

Many people resist the use of genetic engineering to alter organisms. What are their arguments? Do you agree?

Genetics

The Principles of Mendel

Learning Objectives

By the end of this exercise you should be able to:

1. Describe simple genetic dominance, incomplete dominance, and lethal inheritance.
2. Describe possible genotypes for some of your personal traits inherited as dominant and recessive genes.
3. Explain the importance of Mendel's Law of Segregation and Law of Independent Assortment.
4. Distinguish between an organism's phenotype and genotype.



Please visit **connect.mheducation.com** to review online resources tailored to this lab.

Published papers are the primary means of communicating scientific discoveries. One of the most famous of these papers, titled "Experiments in Plant Hybridization," was written in 1866 by Gregor Mendel, an Austrian monk (fig. 17.1). Although Mendel's paper later became the basis for genetics and inheritance, it went largely unnoticed until it was rediscovered independently by several European scientists in 1900. The experiments and conclusions in Mendel's paper now form the foundation of **Mendelian genetics,** the topic of today's exercise.



Figure 17.1 Gregor Mendel (1822–1884) grew and tended pea plants (*Pisum sativum*) like these for his experiments. For each experiment, he observed and counted as many offspring as possible. Pea plants are easy to grow and have many distinct traits; this made it easy for Mendel to analyze many crosses involving lots of offspring. In one set of crosses, he observed and counted a total of 7,324 peas!

Mendel's greatest contribution was to replace the blending theory of inheritance, which stated that all traits blend with each other, with the **particulate theory.** Mendel's particulate theory states that (1) inherited characters are determined by particular factors (now called genes), (2) these factors occur in pairs (i.e., genes occur on maternal and paternal homologous chromosomes), and (3) when gametes form, these genes segregate so that only one of the homologous pair is contained in a particular gamete. Recall from Exercise 15 (Meiosis) that each gamete has an equal chance of possessing either member of a pair of homologous chromosomes. This part of the particulate theory is collectively known as Mendel's First Law, or the **Law of Segregation.** Mendel's Second Law, or the **Law of Independent Assortment,** states that genes on nonhomologous or different chromosomes will be distributed randomly into gametes (figs. 17.2, 17.3).

Mendel's laws describe the inheritance of traits linked to single genes on chromosomes in the nucleus of cells. In today's lab, you'll learn about some of these traits and their inheritance. Remember, however, that not all traits are inherited according to Mendel's laws. For example, several diseases that affect eyes and muscles are inherited from DNA in mitochondria (i.e., not the nucleus). Your textbook discusses this and other types of non-Mendelian inheritance.

Before you start this exercise, briefly review in your textbook some principles and terms pertinent to today's exercise. A **gene** is a unit of heredity on a chromosome. A gene has alternate states called **alleles,** contributed to an organism by its parents. Alleles for a particular gene occur in pairs. Alleles that mask expression of other alleles but are themselves expressed are **dominant;** these alleles are usually designated by a capital letter (for example, *P*). Alleles whose expression is masked by dominant alleles are **recessive** and designated by a lowercase letter (for example, *p*). The **genotype** of an organism includes all the alleles present in the cell, whether they are dominant or recessive. The physical appearance of the trait is the **phenotype.** Thus, if purple flowers (*P*) are dominant to white flowers (*p*), a plant with purple flowers can have a genotype *PP* or *Pp.* A plant with white flowers can only have a genotype *pp* (fig. 17.3). When

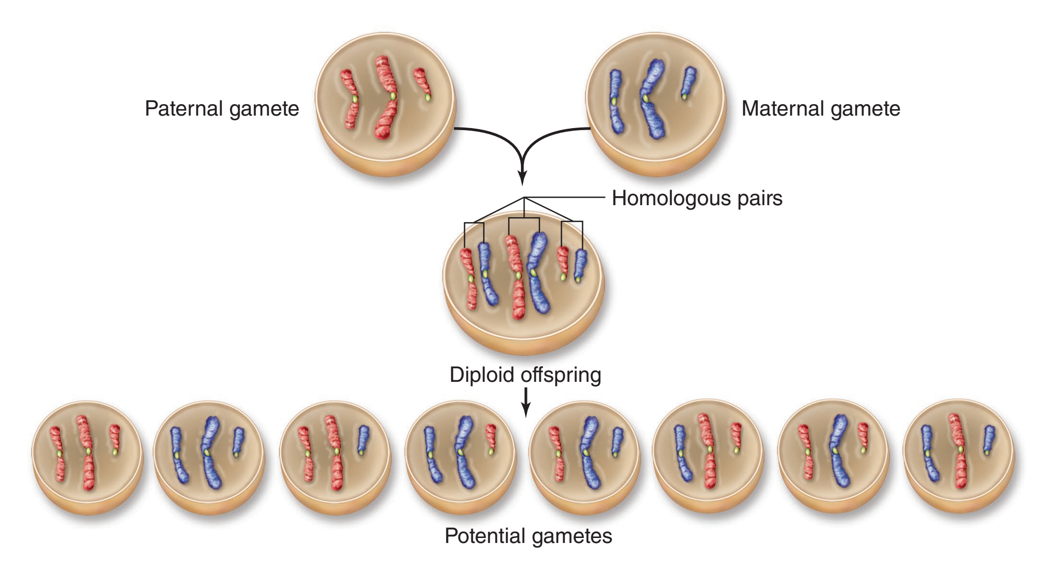
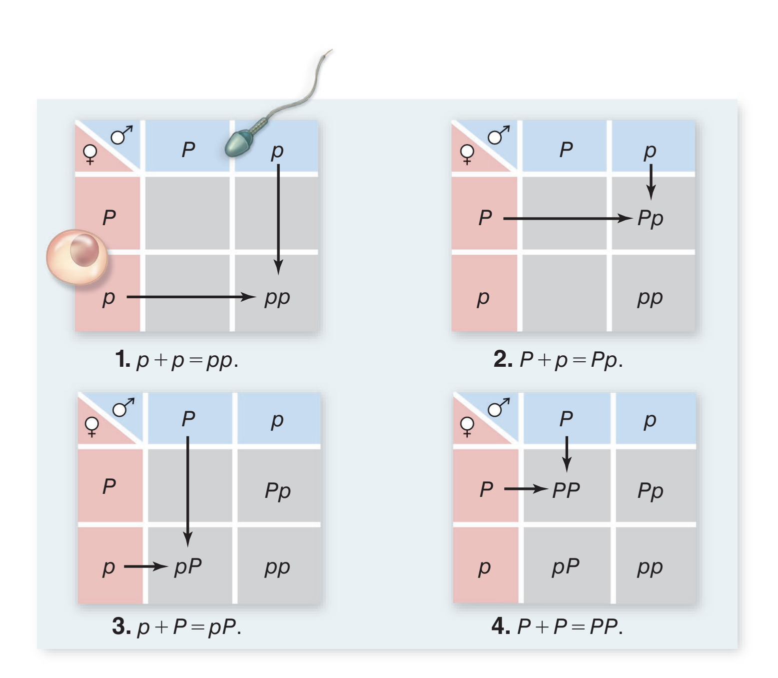


Figure 17.2 Independent assortment increases genetic variability. Independent assortment contributes new gene combinations to the next generation because the orientation of chromosomes on the metaphase plate is random. For example, in cells with three pairs of chromosomes, eight different gametes can result, each with different combinations of parental chromosomes.



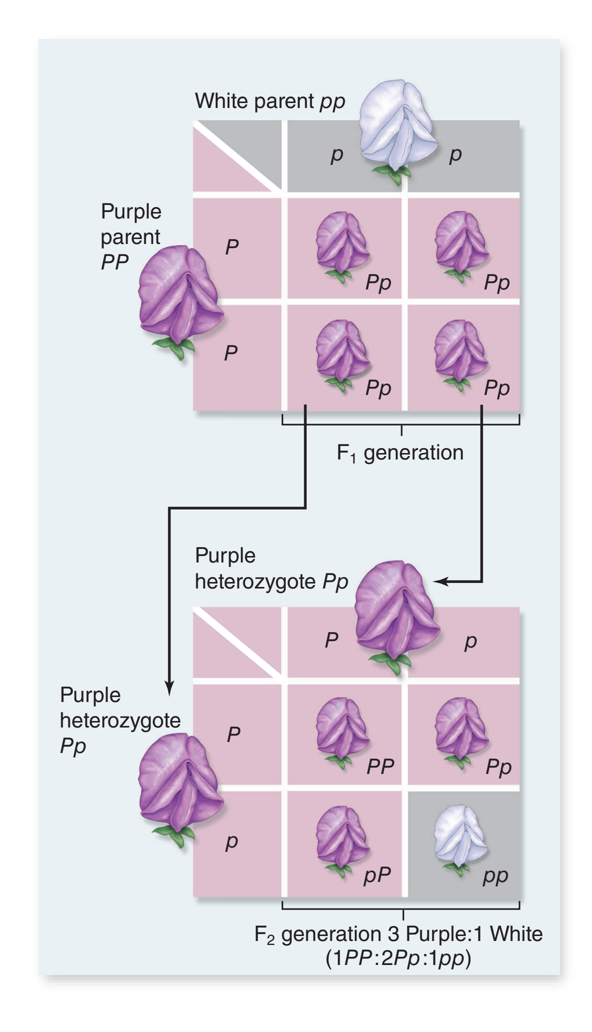
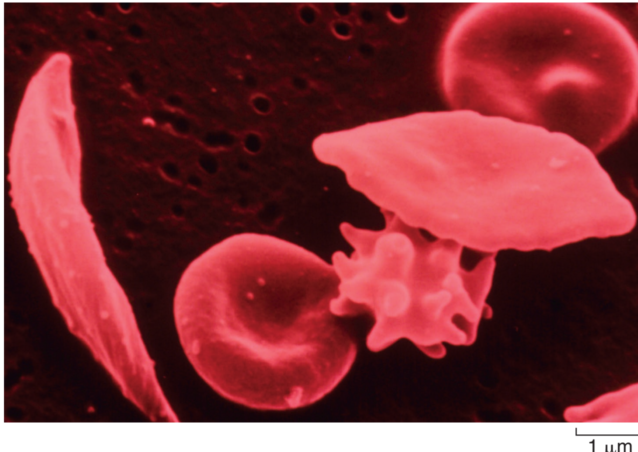


Figure 17.3 In Mendel's crosses of pea plants having purple flowers with plants having white flowers, the original parents each make only one type of gamete. Offspring in the resulting F_1 generation are all *Pp* heterozygotes with purple flowers. These F_1 offspring then each make two types of gametes that can be combined to produce three kinds of F_2 offspring: *PP* homozygotes (purple flowers); *Pp* heterozygotes (also purple flowers); and *pp* homozygotes (white flowers). The ratio of dominant to recessive phenotypes is 3:1. The ratio of genotypes is 1:2:1 (1*PP*:2*Pp*:1*pp*).



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Figure 17.4 Sickle cell anemia. Individuals homozygous for the sickle cell allele have many red blood cells with irregular and sickle shapes, such as the cell on the far left.

the paired alleles are identical (*PP* or *pp*), the genotype is **homozygous** (fig. 17.4). When the paired alleles are different (*Pp*), the genotype is **heterozygous.** With this minimal review, you're prepared to apply this information to solve some genetics problems.

SIMPLE DOMINANCE

Assume that purple flowers are dominant to white flowers. If a homozygous purple-flowered plant is crossed (mated) with a homozygous white-flowered plant, what will be the phenotype (physical appearance) and genotype of the offspring?

Parents: *PP* (homozygous dominant = purple flowers) × *pp* (homozygous recessive = white flowers)

Gametes: *P* from the purple-flowered parent
p from the white-flowered parent

Offspring: genotype = *Pp*
phenotype = purple flowers

This first generation of offspring is called the **first filial** or **F_1 generation** (fig. 17.3).

Each of the F_1 offspring can produce two possible gametes, *P* and *p*. Mendel noted that the gametes from each of the parents combine with each other randomly. Thus, you can simulate the random mating of gametes from the F_1 generation by flipping two coins simultaneously. Assume that heads designates the purple-flower allele (*P*), and tails designates the white-flower allele (*p*). Flipping one coin will determine the type of gamete from one parent and flipping the other will determine the gamete from the other parent. To demonstrate this technique, flip two coins simultaneously 64 times and record the occurrence of each of the three possible combinations in table 17.1.

TABLE 17.1

RESULTS OF COIN-FLIPPING EXPERIMENT SIMULATING RANDOM MATING OF HETEROZYGOUS (*Pp*) INDIVIDUALS

Response	Number
Heads-heads = *PP* = purple flowers	
Heads-tails = *Pp* = purple flowers	
Tails-tails = *pp* = white flowers	

Question 1
What is the ratio of purple-flowered (*PP* or *Pp*) to white-flowered (*pp*) offspring?

Keep these results in mind and return to the original problem: What are the genotypes and phenotypes of the offspring of the F_1 generation?

Parents:	*Pp* × *Pp*	
Gametes:	(*P* or *p*) × (*P* or *p*)	
Offspring genotypes:	*PP Pp pP*	*pp*
Offspring phenotypes:	3 purple	1 white

Thus, the theoretical genotypic ratio for the offspring of the F_1 generation is 1 *PP* : 2 *Pp* : 1 *pp*, and the phenotypic ratio is 3 purple : 1 white.

Question 2

a. How do these ratios compare with your data derived from coin flipping?

b. Would you have expected a closer similarity if you had flipped the coins 64,000 times instead of 64 times? Why or why not?



SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.